

phosphate and three nearly equally charged oxygens (O-11, O-12, O-21) which could partake in ionic binding or hydrogen bonding, or perhaps as general acid-base agents for the ylide protonation deprotonation step. The pyrophosphate charge may also be used in maintaining the Mg(II) metal in its required position.

Further theoretical work on the mechanism of binding as well as a conformational analysis of the dimethylene pyrophosphate side chain will be reported later. The need for further experimental work on the

definition of the solution conformation of the coenzyme as well as on the details of the enzyme-coenzyme-substrate interactions is obvious.

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Topography of Cyclodextrin Inclusion Complexes. III. Crystal and Molecular Structure of Cyclohexaamylose Hexahydrate, the $(\text{H}_2\text{O})_6$ Inclusion Complex¹

Philip C. Manor and Wolfram Saenger*

Contribution from Max-Planck-Institut für Experimentelle Medizin,
Abteilung Chemie, 34 Göttingen, Germany. Received September 29, 1973

Abstract: Cyclohexaamylose, α -cyclodextrin, is a torus-shaped cyclic hexasaccharide consisting of α -(1 \rightarrow 4)-linked glucopyranose residues. Due to the annular aperture 4.5–5.5 Å in diameter in the center of the molecule it is able to form inclusion complexes with a variety of substrate molecules even in aqueous solution. Cyclohexaamylose hexahydrate, the $(\text{H}_2\text{O})_6$ inclusion complex, crystallized from water in the orthorhombic space group $P2_12_12_1$ with cell dimensions $a = 14.856$ Å, $b = 33.991$ Å, $c = 9.517$ Å, and four formula units per unit cell. The crystal structure has been determined on the basis of 4077 observed diffractometer data and refined by the method of least squares to a residual of $R = 6\%$. Four of the six hydration water molecules are located outside the cyclohexaamylose and are part of an extensive hydrogen bonding network. The two remaining water molecules are located within the aperture and almost on the cyclohexaamylose molecular axis. The two water molecules are hydrogen bonded to one another and the water molecule nearer to the O(6) side of the macrocyclic ring is hydrogen bonded to two O(6) hydroxyl groups. The corresponding two C(6)–O(6) bonds are in gauche,trans orientation with respect to the C(5)–O(5) and C(5)–C(4) bonds while the other four C(6)–O(6) bonds are oriented gauche,gauche. The macrocyclic conformation of the cyclohexaamylose torus is less symmetrical and, according to potential energy calculations, is of higher energy than the conformations found with the iodine, 1-propanol, methanol, and potassium acetate adducts. Thus, when the conformation of the cyclodextrin molecule in solution before adduct formation is the same as that found in the case of the hexahydrate here reported, then a conformational change must necessarily be associated with the inclusion process.

The reaction of cyclodextrin glucanotransferase from *Bacillus macerans* with starch yields the cyclodextrins (Schardinger dextrans) which are cyclic oligosaccharides consisting of six or more α -(1 \rightarrow 4)-linked glucopyranose rings. Through the utilization of the 4.5–5.5 Å in diameter void in the center of these torus-shaped molecules, inclusion complexes may be formed both in aqueous solution and in the solid state with the only obvious requirement for complex formation being that the substrate molecule must fit geometrically into the annular void. Eight different nonisomorphous crystalline modifications of α -cyclodextrin (cyclohexaamylose, α -CD) with different guest molecules are known.² The structure we report here is that of α -CD hexahydrate which is approximately isostructural with the I_2 , 1-propanol, methanol, acetic acid, and butyric acid complexes.

As the members of this series of crystalline inclusion complexes are nearly isostructural with one another,

(1) For part II of this series, see R. K. McMullan, W. Saenger, J. Fayos, and D. Mootz, *Carbohydr. Res.*, **31**, 211 (1973).

(2) R. K. McMullan, W. Saenger, J. Fayos, and D. Mootz, *Carbohydr. Res.*, **31**, 37 (1973).

the influence of molecular packing is an almost constant factor. The comparison of the structures of these complexes reveals the influence of the guest molecules on the α -CD conformation. In this regard, the structure of α -CD hexahydrate is particularly interesting as it may represent the structure of the native, "empty" α -CD molecule occurring in aqueous solution and might give a clue as to why α -CD forms inclusion complexes.

This contribution is the third of a series concerned with the structure of cyclodextrin inclusion complexes. A preliminary report of the results of the investigation of the α -CD hexahydrate complex has previously appeared.³ In this paper the details of the structure determination by X-ray crystallographic means and of the molecular geometry of the α -CD molecule will be discussed.

Experimental Section

α -CD was purchased from Corn Products Development, Englewood Cliffs, N. J., and further purified by recrystallization once from 1-propanol and twice from water. Transparent, colorless,

(3) P. C. Manor and W. Saenger, *Nature (London)*, **237**, 392 (1972).

Table I. Fractional Atomic Coordinates and Temperature Factors^a

Atom	X	Y	Z	B ₁₁	B ₂₂	B ₃₃	B ₁₂	B ₁₃	B ₂₃
C 11	.7794(3)	.2316(1)	.0111(6)	164(20)	42(4)	1044(70)	-5(8)	8(34)	4(15)
C 12	.8340(3)	.2165(1)	-.1135(6)	225(21)	47(4)	750(65)	4(8)	9(33)	42(14)
C 12	.7794(2)	.2054(1)	-.2252(4)	313(19)	83(4)	649(45)	16(7)	-101(26)	32(11)
C 13	.8936(3)	.1813(1)	-.0710(6)	159(20)	50(4)	796(65)	0(8)	3(31)	6(14)
C 13	.9584(2)	.1726(1)	-.1755(4)	202(15)	58(3)	732(44)	6(6)	25(23)	-52(10)
C 14	.9449(3)	.1904(1)	.0648(6)	266(22)	27(4)	788(64)	3(8)	-42(33)	-15(13)
C 14	.9878(2)	.1542(1)	.1090(4)	198(14)	37(3)	880(46)	1(5)	5(23)	39(10)
C 15	.8820(3)	.2049(1)	.1782(6)	304(25)	50(4)	779(67)	20(9)	17(35)	-12(15)
C 15	.8350(2)	.2392(1)	.1271(4)	292(16)	35(3)	916(50)	12(6)	-69(25)	-38(10)
C 16	.9279(4)	.2173(2)	.3142(7)	494(34)	76(6)	915(80)	51(11)	-141(44)	-89(18)
C 16	.8594(3)	.2238(1)	.4196(5)	796(33)	78(4)	1083(62)	15(10)	9(39)	-54(14)
C 21	.4504(3)	.1882(1)	.1517(6)	269(22)	36(4)	664(62)	6(8)	10(33)	20(13)
C 22	.4724(3)	.1844(1)	-.0053(5)	214(21)	35(4)	572(56)	0(7)	-76(30)	-18(13)
C 22	.4297(2)	.1492(1)	-.0591(4)	259(16)	45(3)	767(44)	-1(6)	-126(23)	-41(10)
C 23	.5736(3)	.1814(1)	-.0275(5)	230(21)	43(4)	543(58)	10(8)	-11(30)	0(13)
C 23	.5901(2)	.1845(1)	-.1744(4)	305(17)	82(4)	520(41)	-15(7)	69(24)	-55(11)
C 24	.6234(3)	.2137(1)	.0506(5)	182(20)	41(4)	565(57)	0(7)	-2(30)	3(13)
C 24	.7157(2)	.2025(1)	.0445(4)	180(14)	32(3)	950(47)	12(5)	26(23)	17(10)
C 25	.5941(3)	.2176(1)	.2039(5)	228(23)	51(4)	570(60)	-1(8)	-34(31)	-9(13)
C 25	.4965(2)	.2212(1)	.2067(4)	236(15)	38(3)	676(44)	15(5)	20(22)	-26(9)
C 26	.6328(3)	.2535(1)	.2751(6)	316(25)	61(5)	773(68)	0(9)	-99(36)	-55(15)
C 26	.6249(2)	.2888(1)	.1966(4)	317(17)	41(3)	1100(55)	-22(6)	-34(27)	-29(11)
C 31	.4247(3)	.0750(1)	.5276(5)	161(19)	39(4)	698(62)	5(7)	79(30)	-2(13)
C 32	.3725(3)	.0681(1)	.3895(6)	159(19)	34(4)	848(65)	-2(7)	14(31)	0(13)
C 32	.3778(2)	.0270(1)	.3587(4)	253(16)	33(3)	1067(52)	0(6)	-69(25)	-22(10)
C 33	.4156(3)	.0925(1)	.2756(5)	284(23)	38(4)	489(56)	9(8)	-43(32)	-3(12)
C 33	.3626(2)	.0887(1)	.1476(4)	362(18)	49(3)	662(45)	-7(6)	-173(25)	-17(10)
C 34	.4199(3)	.1360(1)	.3189(5)	218(21)	37(4)	621(59)	-16(8)	-3(31)	5(13)
C 34	.4787(2)	.1536(1)	.2174(4)	202(14)	36(3)	682(42)	15(5)	39(21)	45(9)
C 35	.4624(3)	.1407(1)	.4649(5)	214(21)	37(4)	590(59)	0(7)	-37(31)	0(13)
C 35	.4207(2)	.1149(1)	.5661(3)	270(15)	44(3)	535(40)	11(6)	38(22)	-13(9)
C 36	.4537(4)	.1819(1)	.5252(6)	445(29)	50(5)	732(69)	-26(10)	-21(39)	-44(15)
C 36	.3630(2)	.1941(1)	.5395(4)	409(20)	48(3)	946(53)	30(6)	109(28)	-40(11)
C 41	.7307(3)	.0039(1)	.6730(5)	157(20)	41(4)	656(58)	20(7)	-1(30)	13(13)
C 42	.6582(3)	-.0210(1)	.5995(5)	207(21)	37(4)	593(59)	2(8)	3(30)	-5(13)
C 42	.6973(2)	-.0526(1)	.5242(4)	264(16)	43(3)	1049(54)	26(6)	-22(25)	-36(10)
C 43	.6000(3)	.0051(1)	.5092(5)	170(20)	43(4)	480(53)	-12(7)	13(29)	2(12)
C 43	.5281(2)	-.0171(1)	.4488(4)	235(15)	45(3)	601(41)	-15(5)	-85(22)	-21(9)
C 44	.5620(3)	.0390(1)	.5976(5)	202(21)	45(4)	433(54)	1(7)	4(28)	3(13)
C 44	.5158(2)	.0637(1)	.4997(4)	149(13)	52(3)	512(39)	15(5)	-4(20)	35(9)
C 45	.6403(3)	.0619(1)	.6605(5)	164(19)	41(4)	535(56)	11(7)	-39(29)	-17(13)
C 45	.6914(2)	.0356(1)	.7497(3)	175(14)	56(3)	423(38)	16(35)	-26(20)	-5(9)
C 46	.6133(3)	.0970(1)	.7477(6)	308(24)	43(4)	773(66)	8(8)	-45(35)	-59(14)
C 46	.5420(2)	.0874(1)	.8446(4)	368(18)	59(3)	787(47)	38(7)	149(27)	-39(11)
C 51	1.0615(3)	.0475(1)	.5169(6)	238(23)	56(4)	617(61)	10(8)	-23(32)	-1(14)
C 52	1.0400(3)	.0049(1)	.5515(6)	239(22)	54(4)	630(61)	35(8)	43(33)	31(14)
C 52	1.0992(2)	-.0207(1)	.4775(5)	293(18)	57(3)	1261(59)	34(6)	227(28)	54(12)
C 53	.9401(3)	-.0046(1)	.5262(5)	260(23)	38(4)	636(61)	11(8)	47(31)	21(13)
C 53	.9194(2)	-.0438(1)	.5724(4)	330(17)	43(3)	882(48)	0(6)	44(26)	50(10)
C 54	.8811(3)	.0236(1)	.6085(5)	136(19)	47(4)	610(58)	-7(8)	-31(28)	0(13)
C 54	.7894(2)	.0174(1)	.5673(3)	151(14)	50(3)	477(39)	1(5)	-16(20)	3(9)
C 55	.9078(3)	.0663(1)	.5752(6)	208(21)	49(4)	750(65)	-3(8)	-14(32)	-66(14)
C 55	1.0036(2)	.0710(1)	.6906(4)	211(15)	55(3)	636(42)	-15(6)	21(22)	-41(10)
C 56	.8632(4)	.0961(2)	.6694(7)	404(37)	55(5)	1213(86)	-35(10)	182(45)	-121(18)
C 56	.8813(3)	.1348(1)	.6192(5)	455(21)	59(3)	1238(58)	7(7)	-28(31)	-89(12)
C 61	1.0821(3)	.1536(1)	.1221(5)	160(19)	52(4)	622(58)	-7(8)	-23(30)	32(13)
C 62	1.1164(3)	.1153(1)	.0641(5)	248(22)	59(5)	543(58)	-3(9)	9(31)	9(14)
C 62	1.0930(3)	.1114(1)	-.0798(4)	407(19)	86(4)	420(40)	24(7)	32(24)	-10(10)
C 63	1.0787(3)	.0811(1)	.1502(5)	211(20)	43(4)	542(56)	12(8)	-32(30)	-17(13)
C 63	1.1180(2)	.0452(1)	.1019(4)	394(19)	49(3)	714(45)	31(6)	-65(25)	-33(10)
C 64	1.0991(3)	.0857(1)	.3058(5)	182(20)	35(4)	563(57)	-9(7)	-10(28)	0(12)
C 64	1.0460(2)	.0550(1)	.3711(3)	258(15)	33(3)	589(41)	-24(5)	-27(21)	17(8)
C 65	1.0735(3)	.1274(1)	.3587(5)	281(22)	37(4)	617(59)	-3(8)	31(32)	26(12)
C 65	1.1085(2)	.1574(1)	.2637(4)	281(16)	42(3)	698(42)	-22(6)	-23(23)	16(9)
C 66	1.2025(3)	.1340(1)	.4984(5)	392(21)	118(5)	1081(58)	-41(8)	-79(32)	-75(15)
C 66	1.1147(4)	.1394(1)	.5003(6)	371(26)	39(4)	751(65)	-19(9)	-29(37)	-43(14)
WATF01	.2215(3)	.0835(1)	.7415(5)	461(21)	78(4)	957(54)	32(8)	-73(32)	-31(12)
WATF02	.2811(2)	.0041(1)	.7709(4)	272(16)	59(3)	1123(54)	10(6)	59(28)	22(12)
WATF03	.2440(3)	.2184(1)	.3251(6)	584(27)	107(5)	1582(76)	58(9)	101(40)	143(18)
WATF04	.2799(3)	.1631(1)	.7888(5)	397(20)	82(4)	1344(66)	22(8)	-212(32)	-22(14)
WATF05	.7356(4)	.1573(2)	.4622(6)	709(33)	190(7)	1656(88)	-3(13)	-380(46)	115(22)
WATF06	.7043(5)	.1007(2)	.2349(8)	1128(47)	194(9)	2524(128)	89(17)	-233(75)	-232(29)

^a See legend to Table II.

Table II. Fractional Atomic Coordinates and Temperature Factors^a

Atom	X	Y	Z	B	Atom	X	Y	Z	B
HC11	0.747	0.257	-0.019	2.31	HC61	1.110	0.176	0.066	1.90
HC12	0.875	0.239	-0.145	2.23	HC62	1.183	0.115	0.072	2.08
HC13	0.853	0.157	-0.053	2.03	HC63	1.012	0.080	0.138	1.77
HC14	0.992	0.211	0.044	1.98	HC64	1.163	0.081	0.322	1.61
HC15	0.836	0.184	0.200	2.43	HC65	1.008	0.130	0.367	1.85
HC16	0.969	0.197	0.345	3.59	HC66	1.091	0.123	0.577	2.42
HC16	0.961	0.243	0.297	3.59	HC66	1.104	0.168	0.518	2.42
HC21	0.384	0.192	0.161	2.06	HO12	0.740	0.214	-0.300	2.80
HC22	0.449	0.209	-0.056	1.74	HO13	0.975	0.158	-0.225	2.19
HC23	0.596	0.156	0.010	1.91	HO16	0.850	0.251	0.415	4.42
HC24	0.613	0.239	0.001	1.65	HO22	0.375	0.155	-0.110	2.32
HC25	0.613	0.193	0.258	2.00	HO23	0.660	0.186	-0.200	2.71
HC26	0.599	0.258	0.366	2.61	HO26	0.575	0.295	0.150	2.62
HC26	0.697	0.250	0.297	2.61	HO32	0.310	0.019	0.345	2.42
					HO33	0.395	0.100	0.100	2.43
HC31	0.399	0.058	0.601	1.81	HO36	0.305	0.195	0.575	2.95
HC32	0.308	0.075	0.404	1.78	HO42	0.750	-0.046	0.488	2.47
HC33	0.478	0.084	0.256	1.82	HO43	0.500	0.000	0.388	2.02
HC34	0.358	0.148	0.317	1.78	HO46	0.503	0.110	0.882	2.69
HC35	0.528	0.134	0.459	1.88	HO52	1.085	-0.025	0.400	3.06
HC36	0.482	0.183	0.619	2.87	HO53	0.950	-0.041	0.630	2.58
HC36	0.485	0.201	0.461	2.87	HO56	0.900	0.137	0.550	3.41
					HO62	1.065	0.095	-0.160	2.77
HC41	0.766	-0.014	0.740	1.78	HO63	1.055	0.034	0.025	2.54
HC42	0.617	-0.033	0.675	1.81	HO66	1.230	0.130	0.425	3.93
HC43	0.639	0.016	0.433	1.57					
HC44	0.522	0.030	0.674	1.64	H2O1	0.245	0.056	0.735	3.37
HC45	0.681	0.071	0.582	1.61	H2O1'	0.270	0.077	0.785	3.37
HC46	0.668	0.107	0.800	2.33	H2O2	0.330	-0.008	0.715	2.70
HC46	0.594	0.119	0.680	2.33	H2O2'	0.270	-0.010	0.815	2.70
					H2O3	0.280	0.206	0.395	4.78
HC51	1.127	0.053	0.537	2.22	H2O3'	0.210	0.227	0.368	4.78
HC52	1.054	0.000	0.653	2.09	H2O4	0.225	0.179	0.750	3.79
HC53	0.926	-0.002	0.424	2.05	H2O4'	0.270	0.158	0.865	3.79
HC54	0.890	0.020	0.710	1.69	H2OA	0.780	0.150	0.490	6.30
HC55	0.893	0.072	0.473	2.01	H2OA'	0.695	0.146	0.400	6.30
HC56	0.877	0.094	0.771	3.46	H2OB	0.685	0.115	0.175	7.98
HC56	0.790	0.094	0.666	3.46	H2OB'	0.710	0.090	0.325	7.98

^a Anisotropic temperature factors are multiplied by 10^3 and are in the form $T = \exp[-(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + 2B_{12}hk + 2B_{13}hl + 2B_{23}kl)]$. Estimated standard deviations are given in parentheses. Hydrogen atoms are estimated from the difference Fourier to ± 0.003 (fractional). The atoms are designated as, for example, C(51) meaning atom C(1) in glucose number 5. The glucose numbers 1-6 follow clockwise when the molecule is viewed from the O(2)-O(3) side (Figure 1).

prismatic crystals were obtained through the slow cooling of a saturated aqueous solution of α -CD. Weissenberg and precession method photographic data indicate that the space group is orthorhombic with systematic extinctions corresponding uniquely to space group $P2_12_12_1$ (D_2^4 , International Tables, No. 19). The unit cell dimensions and estimated standard deviations, $a = 14.856$ (3), $b = 33.991$ (13), $c = 9.517$ (5) Å, were determined at room temperature using Ni-filtered Cu radiation ($\lambda_{\text{mean}} = 1.5418$ Å) from nine diffractometer-measured 2θ data and refined by the method of least squares. With four units of formula $(C_6H_{10}O_5)_6 \cdot 6H_2O$ per cell, formula wt 1081 Daltons, the calculated density is 1.493 g/cm³. The density observed using the flotation method with cyclohexane and methyl iodide is 1.491 g/cm³.

Up to a 2θ limiting value of 130° , a total of 4558 reflection intensities were measured using a Siemens automatic diffractometer. Of this number, the 481 reflections with measured intensities less than 3σ above background (σ estimated from counting statistics plus 3% of I_{net})⁴ were eliminated from the data set used for the structure refinement. The θ - 2θ scan method was utilized with stationary background measurements both at the beginning and end of each scan. Only background, Lorentz, and polarization corrections were applied to the data.

It was initially expected that the structure of α -CD hexahydrate would be sufficiently isomorphous with the structure of the I_2 inclusion complex, which had been previously determined by the heavy-atom method,⁵ so that the coordinates of the latter could be directly applied to the former. This ultimately proved to be the

case when the O(6) and water atoms were ignored and when only the 977 α -CD hexahydrate reflection intensities with $2\theta \leq 60^\circ$ and/or $F_{\text{obsd}} \geq 75$ were used to adjust the atomic coordinates in the initial least-squares refinement cycles.

The final least-squares method of structure refinement was carried out using a modified version of the program ORFLS⁶ which allowed a "fixed atom" contribution from the 42 hydrogen atoms whose positions could be calculated and the 30 hydroxyl and water hydrogen atoms whose positions could be estimated from a difference Fourier synthesis. The isotropic temperature factor for each hydrogen atom was computed from the mean of the diagonal elements of the anisotropic temperature factor matrix of the atom to which the hydrogen atom was bound. No attempt was made to refine hydrogen atom positions or temperature factors. All other atomic coordinates were refined along with anisotropic thermal parameters for each atom. The refinement was carried out in blocks corresponding to the coordinates and associated thermal parameters of 20 atoms; the atoms included in each block were varied from cycle to cycle with atoms bound to one another confined as far as possible to the same block. The final disagreement indices are $R_1 = \sum |F_o| - |SF_c| / \sum |F_o| = 0.060$ and $R_2 = (\sum w \cdot (|F_o| - |SF_c|)^2 / \sum w F_o^2)^{1/2} = 0.047$ with the weight corresponding to $1/\sigma$ previously defined and with the sums taken over all reflections. Throughout the refinement the quantity minimized was $\sum w (|F_o| - |SF_c|)^2$. The atomic scattering factors were taken from the International Tables for X-ray crystallography.⁷ The final coordinates

(6) W. R. Busing, K. O. Martin, and H. A. Levy, "ORFLS, A Fortran Crystallographic Least-Squares Program," USAEC Report ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1962.

(7) "International Tables for X-Ray Crystallography," Vol. III, Kynoch Press, Birmingham, England, 1962, pp 202-203.

(4) H. G. Stout and L. H. Jensen, "X-Ray Structure Determination," Macmillan, London, 1968, p 457.

(5) R. K. McMullan, W. Saenger, J. Fayos, and D. Mootz, *Carbohydr. Res.*, 31, 211 (1973).

Table III. α -Cyclodextrin Hexahydrate Bond Lengths (\AA)^a

Atom	Glucose unit						Mean, y_i	σ_i	
	1	2	3	4	5	6			
C(1)-C(2)	1.5410	1.5343	1.5440	1.5380	1.5193	1.5051	1.5303	.015	$S_b = 0.00105$ $S_w = 0.00397$ $F_w = 1.645$ Prob > 80%
C(2)-C(3)	1.5254	1.5218	1.5078	1.5073	1.5375	1.5285	1.5214	.012	
C(3)-C(4)	1.5321	1.5188	1.5361	1.5078	1.5181	1.5188	1.5220	.010	
C(4)-C(5)	1.5140	1.5281	1.5350	1.5334	1.5351	1.5534	1.5326	.014	
C(5)-C(6)	1.5222	1.5108	1.5203	1.5064	1.5059	1.5348	1.5167	.011	
mean	1.5262	1.5228	1.5286	1.5185	1.5232	1.5281	1.5246 \AA		
σ_i	.011	.009	.014	.016	.013	.018			
	$S_b = 0.00036, S_w = 0.00465, F = 0.376, \text{Prob} < 50\%$								
C(1)-O(5)	1.4029	1.4173	1.4075	1.4260	1.4184	1.4096	1.4136	.008	$S_b = 0.00601$ $S_w = 0.00478$ $F_w = 7.337$ Prob > 99.5%
C(1)-O(4)	1.4037	1.3952	1.4308	1.4080	1.4284	1.4063	1.4121	.014	
C(2)-O(2)	1.4063	1.4467	1.4299	1.4178	1.4235	1.4190	1.4239	.014	
C(3)-O(3)	1.4154	1.4238	1.4569	1.4296	1.4369	1.4282	1.4318	.014	
C(4)-O(4)	1.4494	1.4235	1.4340	1.4301	1.4328	1.4485	1.4364	.010	
C(5)-O(5)	1.4428	1.4556	1.4418	1.4485	1.4532	1.4578	1.4490	.007	
C(6)-O(6)	1.4456	1.4186	1.4163	1.4411	1.4263	1.4207	1.4281	.012	
mean	1.4237	1.4258	1.4310	1.4287	1.4313	1.4263	1.4278 \AA		
σ_i	.021	.020	.016	.013	.011	.019			
	$S_b = 0.00033, S_w = 0.01046, F = 0.225, \text{Prob} < 50\%$								

^a Observations: $y_{ij}, i = 1, n; j = 1, m; n = \text{number of classes}; m = \text{number of observations in each class}; S_w = \sum_i \sum_j (y_{ij} - \bar{y}_i)^2; S_b = m \sum_i (\bar{y}_i - \bar{y})^2; F = (S_b/n - 1)/(S_w/mn - n); \sigma_i = [\sum_j (y_{ij} - \bar{y}_i)^2/m - 1]^{1/2}$.

Table IV. α -Cyclodextrin Hexahydrate Bond Angles (deg)

Atoms involved	Glucose unit						Mean, y_i	σ_i	
	1	2	3	4	5	6			
C(1)-C(2)-C(3)	111.28	110.58	108.34	109.72	111.68	109.78	110.23	1.2	$S_b = 60.29$ $S_w = 25.49$ $F_w = 15.76$ Prob > 99.5%
C(2)-C(3)-C(4)	110.09	111.37	110.71	109.90	110.08	111.80	110.66	0.8	
C(3)-C(4)-C(5)	111.18	113.00	111.15	108.64	109.97	111.21	110.85	1.5	
C(4)-C(5)-C(6)	114.85	112.95	113.77	114.71	113.50	115.45	114.22	0.9	
mean	111.85	111.98	110.99	110.74	111.31	112.06	111.48		
σ_i	2.1	1.2	2.2	2.7	1.7	2.4			
	$S_b = 6.11, S_w = 79.67, F = 0.276, \text{Prob} < 50\%$								
C(2)-C(1)-O(5)	110.30	108.86	110.33	111.14	106.72	109.56	109.44	1.5	$S_b = 263.9$ $S_w = 179.8$ $F_w = 8.07$ Prob > 99.5%
C(2)-C(1)-O(4)	107.96	107.52	106.05	106.77	110.23	108.47	107.83	1.4	
C(1)-C(2)-O(2)	110.18	108.68	107.18	111.04	110.22	110.56	109.64	1.4	
C(3)-C(2)-O(2)	109.07	109.18	111.47	113.19	112.94	110.93	111.13	1.8	
C(2)-C(3)-O(3)	111.08	107.53	108.85	110.23	110.78	109.10	109.60	1.3	
C(4)-C(3)-O(3)	107.28	110.02	109.39	110.10	107.75	108.72	108.88	1.2	
C(3)-C(4)-O(4)	106.93	104.86	104.32	105.09	108.35	103.65	105.53	1.8	
C(5)-C(4)-O(4)	110.01	109.58	108.40	108.89	109.19	112.71	109.80	1.5	
C(4)-C(5)-O(5)	108.82	107.94	111.37	108.37	108.92	110.48	109.32	1.3	
C(6)-C(5)-O(5)	106.31	107.56	105.72	107.53	104.87	102.53	105.75	1.9	
C(5)-C(6)-O(6)	108.51	114.57	112.73	111.65	118.38	110.50	112.72	3.4	
mean	108.79	108.75	108.71	109.45	109.82	108.83	109.05		
σ_i	1.5	2.4	2.7	2.4	3.5	3.1			
	$S_b = 11.97, S_w = 431.75, F = 0.33, \text{Prob} < 50\%$								
C(4)-O(4)-C(1)	118.40	118.16	118.04	119.41	116.20	117.19	117.9	1.1	
C(1)-O(5)-C(5)	113.54	114.05	113.13	112.45	115.93	115.49	114.1	1.4	
O(5)-C(1)-O(4)	110.46	110.84	110.30	111.89	110.40	111.16	110.8	0.6	

and thermal parameters are given in Tables I and II; observed and calculated structure factors are given in the supplementary material.⁸ One α -CD molecule, viewed parallel and perpendicular to the line connecting the two water molecules, is shown in Figure 1 and the geometric data are presented in Tables III-VII.

Results and Discussion

Glucopyranose Conformation. Bond Lengths and Angles. In the first instance the structure of α -CD may

(8) See paragraph at end of paper regarding supplementary material.

be considered as six independent observations of glucopyranose rings in the C(1) chair conformation. The α -CD bond lengths and angles computed from the entries of Table I are given in Tables III and IV. Also indicated in Tables III and IV are the results of the F ratio statistical test⁹ which indicates that there are no significant differences in corresponding glucopyranose

(9) W. C. Hamilton, "Statistics in Physical Science," Ronald Press, New York, N. Y., 1964, pp 96-102.

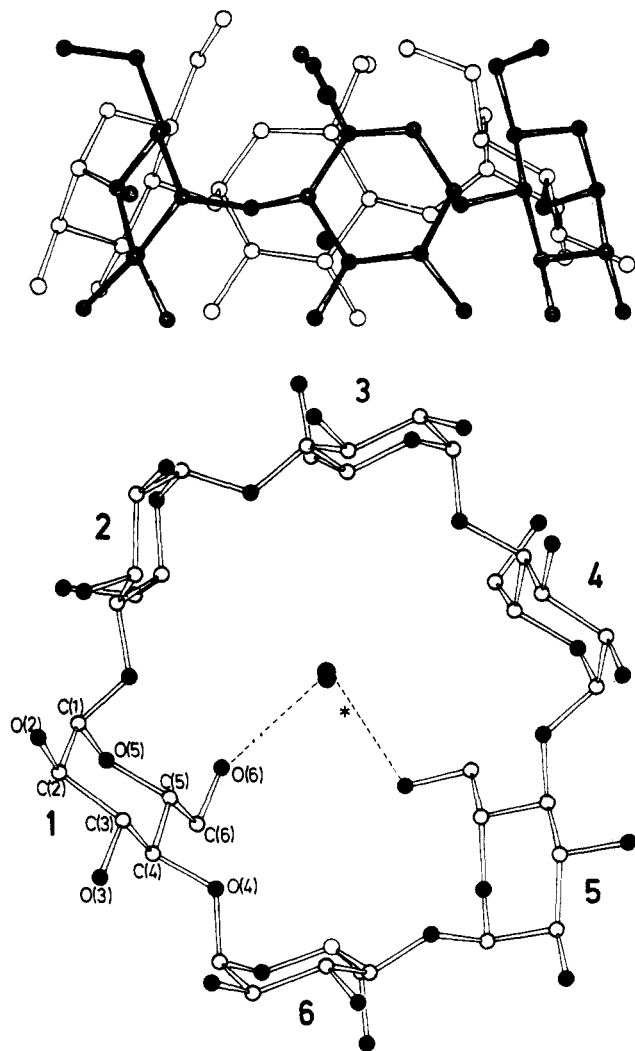


Figure 1. α -CD parallel and perpendicular to the line connecting the two water molecules. The asterisk indicates the approximate center of the O(4) atoms.

bond lengths or angles when any one of the six glucopyranose rings is compared with the other remaining five. The mean bond lengths and angles may therefore be considered as meaningful and suitable for comparison with other glucose monomer and dimer structures. Further, it is apparent from Table III that the C–C (C–O) bond lengths are specifically dependent on the chemical nature of the atoms involved. In particular, the C(5)–O(5) mean bond length is 0.021 Å longer than the mean overall C–O distance (1.428 Å), and the C(1)–O(5) and C(1)–O(4) bonds are each approximately 0.015 Å shorter than the mean. These differences are similar to those found in other diffraction studies involving the glucopyranose ring¹⁰ and with reported anomeric effect theoretical calculations.¹¹

The comparison of the average glucopyranose from α -cyclodextrin hexahydrate with monomeric α -D-glucose illustrates the seeming mutability of glucose geometry under variable external influence. Two highly refined monomer diffraction studies have been reported: one the structure of an anhydrous, ortho-

(10) H. M. Berman, S. S. C. Chu, and G. A. Jeffrey, *Science*, **157**, 1576 (1967).

(11) G. A. Jeffrey, J. A. Pople, and L. Radom, *Carbohydr. Res.*, **25**, 117 (1973).

Table V. Glucosidic Bond Conformation and O(2)–O(3) Distances

Atoms involved				Torsion angle, deg	
O	C	O	C	ϕ_1	
15	11	24	24	112.8	
25	21	34	34	104.8	
35	31	44	44	107.5	
45	41	54	54	88.2	
55	51	64	64	90.4	
65	61	14	14	100.7	
C	C	O	C	ϕ_1'	
12	11	24	24	-126.5	
22	21	34	34	-136.7	
32	31	44	44	-133.0	
42	41	54	54	-150.0	
52	51	64	64	-151.9	
62	61	14	14	-138.8	
C	O	C	C	ϕ_2	
11	24	24	23	135.3	
21	34	34	33	131.0	
31	44	44	43	128.4	
41	54	54	53	116.6	
51	64	64	63	170.4	
61	14	14	13	120.6	
C	O	C	C	ϕ_2'	
11	24	24	25	-103.2	
21	34	34	35	-110.5	
31	44	44	45	-115.4	
41	54	54	55	-123.7	
51	64	64	65	-69.3	
61	14	14	15	-118.5	
O	C	O	C	TA ^a	ϕ
24	21	34	34	162.6	-17.4
34	31	44	44	165.9	-14.1
44	41	54	54	147.6	-32.4
54	51	64	64	147.4	-32.6
64	61	14	14	160.9	-19.1
14	11	24	24	171.1	-8.9
C	O	C	O	TA	ϕ'
21	34	34	44	-169.9	190.1
31	44	44	54	-172.9	187.1
41	54	54	64	178.6	178.6
51	64	64	14	-131.2	228.8
61	14	14	24	-175.8	184.2
11	24	24	34	-162.6	197.4
Atoms				Distance, Å	
O	O				
12	23			2.941	
22	33			3.016	
32	43			2.823	
42	53			3.345	
52	63			4.666	
62	13			3.025	

^a The values listed under TA conform to the standard definition of the torsion angle (ref 14) and the values listed under ϕ and ϕ' conform to the definition given in ref 19.

rhombic crystalline form studied by neutron diffraction¹² and the other a monoclinic, monohydrate form investigated in a precision X-ray diffraction study¹³ (Figure 2). In both studies the standard deviations in bond lengths for C–C and C–O bonds are in the range 0.002–0.006 Å, and there are differences of as much as 0.024 Å between corresponding bond lengths in the two structures. When the C–C bond lengths are considered, the differences between the results of the three studies are small; however, more variation is found with regard to C–O bond lengths. The C–O bond lengths

(12) G. M. Brown and H. A. Levy, *Science*, **147**, 1038 (1965).

(13) E. Hough, S. Neidle, D. Rogers, and P. G. H. Troughton, *Acta Crystallogr., Sect. B*, in press.

Table VI. Least-Squares Planes

I. Deviations from the Mean Plane of Atoms C(2), C(3), C(5), and O(5), Å

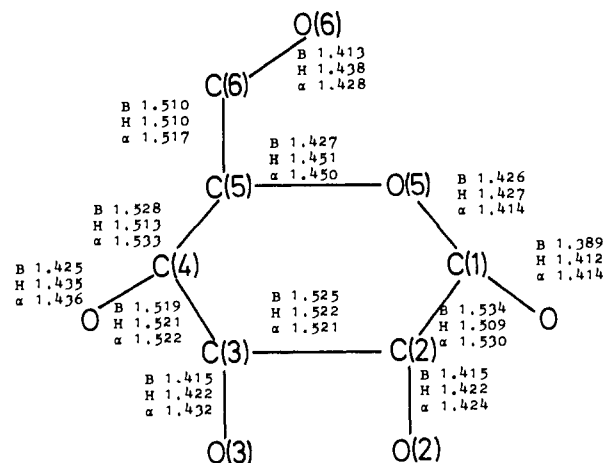
Atom	Glucose					
	1	2	3	4	5	6
C(1)	-0.648	-0.687	0.693	0.653	-0.689	-0.675
C(2)	-0.032	-0.029	-0.007	0.020	-0.011	0.005
C(3)	0.031	0.027	0.006	-0.019	0.010	-0.005
C(4)	0.659	0.612	-0.629	-0.725	0.667	0.606
C(5)	-0.033	-0.028	-0.007	0.020	-0.011	0.005
O(5)	0.034	0.030	0.007	-0.020	0.012	-0.005

II. Deviations of the Oxygen Atoms, O(4), Linking Glucose from the Mean Plane of All O(4) Atoms, Å

Glucose	O(4) deviation	C(1) deviation	C(4) deviation
1→2	0.092	0.589	0.206
2→3	-0.136	0.076	0.116
3→4	0.057	0.335	0.324
4→5	0.057	0.428	0.659
5→6	-0.094	0.836	0.187
6→1	0.023	0.279	0.405

III. Deviations of the O(2) and O(3) Atoms from the Mean Plane of All O(2) and O(3) atoms, Å

Glucose	O(2)	O(3)
1	0.394	0.173
2	-0.067	0.086
3	0.033	-0.060
4	-0.299	-0.188
5	0.501	0.461
6	-0.414	-0.621

Figure 2. Glucopyranose ring bond lengths B from Brown and Levy,¹² H from Hough, Neidle, Rogers, and Troughton,¹³ and α the mean α -CD hexahydrate bond lengths.

The situation of O(5) relative to C(5) and C(1) is different in the neutron and X-ray diffraction studies. In the anhydrous orthorhombic crystals studied with neutron diffraction these distances are equal to within experimental error: 1.427 and 1.426 Å. In the mono-

Table VII. Dihedral Angles

Angle	I. Glucopyranose Ring Dihedral Angles					
	1	2	3	4	5	6
C(N1)-C(N2)-C(N3)-C(N4)	-49.3	-49.9	-55.1	-54.0	-55.3	-54.6
C(N2)-C(N3)-C(N4)-C(N5)	52.0	49.0	51.2	58.1	52.8	49.1
C(N3)-C(N4)-C(N5)-O(N5)	-57.2	-52.3	-50.3	-60.9	-54.1	-47.6
C(N4)-C(N5)-O(N5)-C(N1)	63.4	61.8	57.3	63.2	61.8	55.8
C(N5)-O(N5)-C(N1)-C(N2)	-61.3	-64.6	-62.6	-59.9	-62.1	-61.8
O(N5)-C(N1)-C(N2)-C(N3)	53.5	56.6	60.8	54.5	57.0	59.1
	II. Atom O(6) Relative to O(5) and C(4)					
Glucose	O(5)-C(5)-C(6)-O(6)			C(4)-C(5)-C(6)-O(6)		
1	69.3° gauche			-170.2° trans		
2	-71.8° gauche			47.1° gauche		
3	-63.1° gauche			59.3° gauche		
4	-70.1° gauche			46.7° gauche		
5	69.7° gauche			-171.7° trans		
6	-67.6° gauche			52.5° gauche		

when oxygen is hydroxyl are, as expected, consistently shorter in the structure determined by neutron diffraction methods than in either of the structures examined by X-ray diffraction; the average differences between the neutron structure and the mean of the X-ray results is 0.010 Å. The bonds making up the link between glucopyranose rings, C(1)-O(4) and C(4)-O(4), are of the same length in the α -CD average glucose as the corresponding bonds in the α -D-glucose monohydrate structure determined by X-ray methods: C(1)-O(4) = 1.414, 1.412 Å and C(4)-O(4) = 1.436, 1.435 Å in α -CD and α -D-glucose monohydrate, respectively. The C(4)-O(4) distances in α -CD are without exception greater than the associated C(1)-O(4) distances, and there does not appear to be any general rule with regard to the relative lengths of the C(1)-O(4) and C(1)-O(5) bonds. The macrocyclic restrictions and oxygen substitutions have no apparent effect on the C(1)-O(4) or O(4)-C(4) bond lengths in α -cyclodextrin.

clinic monohydrate form determined by X-ray methods C(1)-O(5) remains essentially unchanged at 1.427 Å. However, C(5)-O(5) is considerably longer, 1.451 Å. In α -CD, the average glucopyranose C(5)-O(5) bond length is "long," 1.450 Å, and C(1)-O(5) had become somewhat shorter, 1.414 Å.

The angles within the glucopyranose unit, Table IV, illustrate the same consistency shown by the bond distances with two exceptions. In glucopyranoses ring number 5 the C(5)-C(6)-O(6) angle is 118.4° and is thus significantly larger than the other C(5)-C(6)-O(6) angles. This is presumably associated with the hydrogen bonding of the O(56) hydroxyl group to one of the included water molecules. The C(5)-C(6)-O(6) angle of glucopyranose ring number 1, 108.4°, is the smallest such angle and this O(16) hydroxyl group is also assumed to be involved in hydrogen bonding to the included water.

The C(1) chair conformation of the individual glucopyranose is demonstrated by the deviations from the

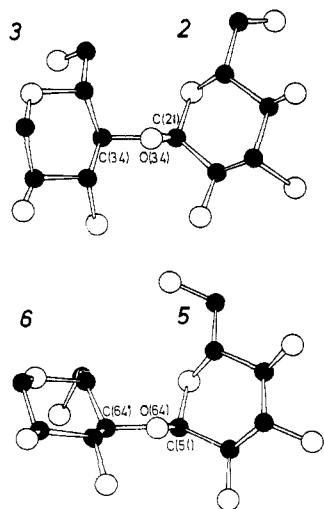


Figure 3. Glucopyranoses 2, 3 and 5, 6 seen from the inside of the α -CD torus normal to the C(4)-O(4) bond. The C(4)-O(4)-C(1) plane is normal to the page.

least-squares plane of atoms C(2), C(3), C(5), and O(5), Table VI, and by the endocyclic torsion angles¹⁴ about each of the bonds of the glucopyranose ring, Table VII. In every case atoms C(2), C(3), C(5), and O(5) lie within ± 0.034 Å of their common mean plane and the atoms C(1) and C(4) are each displaced by as much as 0.725 Å toward opposite sides of this plane. The torsion angles show more clearly that within the restrictions of the C(1) conformation a certain amount of conformational freedom is possible, with a range of 13.3° in the angle C(3)-C(4)-C(5)-O(5). The other torsion angles are more constant with a range of 9° .

Macrocyclic Ring Conformation. The conformation of the C(1)-O(4)-C(4) linkage between the six glucopyranoses defines the macrocyclic conformation of the cyclodextrin ring. The parameters specifying this linkage—the two bond distances, the bond angle, and the torsion angles for rotations about the C-O bonds—reflect the size and nature of the included substance and the intermolecular packing forces. They are the principal parameters specifying the differences between different cyclodextrin inclusion complexes, and it is primarily with regard to the rotation parameters that the structure of α -CD hexahydrate differs from the nominally isomorphous 1-propanol, I₂, methanol, and other inclusion complexes.

The C(1)-O(4) and C(4)-O(4) bond lengths and the C(1)-O(4)-C(4) bond angles are not subject to major variation. In α -CD hexahydrate the individual C-O bond lengths between glucopyranoses show no greater variability than the other C-O bond lengths (Table III). The bond angle (Table V) appears to be confined to a narrow range $118 \pm 2^\circ$. For comparison, in the nonisomorphous potassium acetate inclusion com-

plex,¹⁶ the C(1)-O(4)-C(4) angles are 119.1, 119.4, and 118.8° (mean est std dev 0.8°) and in the disaccharide β -maltose monohydrate¹⁷ the angle is $117(1)^\circ$.

In contrast to all of the other α -CD inclusion complexes thus far investigated, α -cyclodextrin hexahydrate shows a major anomaly with regard to the rotation parameters about the bonds of the glucosidic linkage. Quantitatively, the rotation parameters specifying the orientation of one glucose relative to the next are defined according to two systems. In the first,¹⁸ the angles ϕ_1 , ϕ_1' , ϕ_2 , ϕ_2' specify respectively the torsion angles O(5)-C(1)-O(4')-C(4'), C(2)-C(1)-O(4')-C(4'), C(1)-O(4')-C(4')-C(3'), and C(1)-O(4')-C(4')-C(5') (primed and unprimed atoms designate atoms of adjacent glucopyranose). In the second method¹⁹ the angles ϕ and ϕ' involve "virtual" O(4)-C(1) bonds and designate, in cyclodextrin, the torsion angles O(n,4) \cdots C(n,1)-O(n+1,4)-C(n+1,4) and C(n,1)-O(n+1,4)-C(n+1,4) \cdots O(n+2,4). This second method is of advantage for the description of the overall polymer conformation and for comparison with published potential energy calculations. The values for these angles in α -CD hexahydrate are given in Table V.

It is apparent that in α -CD hexahydrate one of the glucose residues, number 5, is rotated in such a way that this glucose ring is more nearly normal to the axis of the α -CD torus than the other five glucoses, figure 5, Table V. The atom C(51) is nearly trans to C(63); the dihedral angle C(51)-O(64)-C(64)-C(63) is 170.4° instead of the 120° characteristic of a linear polysaccharide chain. This anomalous linkage between glucose residues numbers 5 and 6 is shown in Figure 3 along with one of the more "normal" linkages.

According to the calculations of Rao, *et al.*,¹⁹ the anomalous linkage between glucose residues 5 and 6 corresponds to a high-energy conformation. In Figure 4 the values of ϕ and ϕ' found for α -CD hexahydrate are plotted on the conformational energy maps due to these authors. The point at $\phi = -32.6^\circ$, $\phi' = 228.8^\circ$ is the linkage between glucose residues 5 and 6 and corresponds to an increased energy of about 1.5 kcal/mol relative to the energy minimum when the nonbonded intermolecular potentials are computed using the Kitaigorodskii potential function: $V = 3.5(-0.04/Z^8 + 8.6 \times 10^3 e^{-13Z})$, $Z = r_{ij}/r_0$, $r_0 =$ equilibrium distance between the interacting atoms i and j . The same point corresponds to an increase in potential energy of 6 kcal/mol when the interactions between nonbonded atoms are computed using the alternative Buckingham form potential function with parameters due to Flory: $V = -a/r_{ij}^6 + Ce^{-\mu r_{ij}}$.

Two factors are presumably involved in compensating for the relatively high energy of this conformation: (1) the formation of hydrogen bonds between the included water and two cyclodextrin O(6) hydroxyl groups and (2) the reduction of the "empty volume" of the cyclodextrin ring. In α -CD hexahydrate the C(6)-O(6) bonds of glucose residues 1 and 5 are in

(14) The torsion angle for the four-atom group A-B-C-D is defined as the angle between the bonds A-B and C-D when viewed in projection along bond C-B. According to the IUPAC-IUB¹⁵ rules for the assignment of sign and zero: (1) when the bond A-B is cis-coplanar with the bond C-D the torsion angle is zero; (2) when A-B-C-D is viewed along C-B with A, the point furthest away, and A is rotated clockwise relative to the stationary point D the torsion angle is positive; and (3) the absolute magnitude of the torsion angle is $\leq 180^\circ$.

(15) *Biochim. Biophys. Acta*, **229**, 1 (1971); see also *J. Mol. Biol.*, **52**, 1 (1970).

(16) A. Hybl, R. E. Rundle, and D. E. Williams, *J. Amer. Chem. Soc.*, **87**, 2779 (1965).

(17) G. J. Quigley, A. Sarko, and R. H. Marchessault, *J. Amer. Chem. Soc.*, **92**, 5834 (1970).

(18) M. Sundaralingam, *Biopolymers*, **6**, 189 (1968).

(19) V. S. R. Rao, P. R. Sundararajan, C. Ramakrishnan, and G. N. Ramachandran, "Conformation of Biopolymers," Vol. 2, G. N. Ramachandran, Ed., Academic Press, London and New York, 1967, pp 721-737.

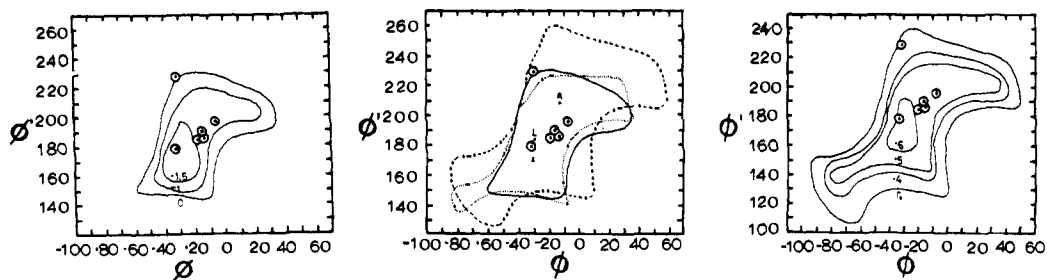


Figure 4. α -CD hexahydrate glucosidic bond conformation plotted on the conformational energy maps due to Rao, *et al.*¹⁹ Left: energy contours in kilocalories per mole computed using the Kitaigorodskii function. Center: zero energy (---), Flory function; outer limit contour (····); zero energy computer using Kitaigorodskii potential function (—). Right: energy contours in kilocalories per mole computed using the Flory function.

the gauche,trans conformation relative to bonds C(5)–O(5) and C(5)–C(4); *i.e.*, they are directed toward the center of the cyclodextrin ring and are in an orientation such that the formation of hydrogen bonds to the included water is possible (Figure 1). The water molecules of van der Waals diameter 3.6 Å are not located at the center of the molecule on the axis normal to the plane of and equidistant from the O(4) atoms, but rather they are displaced approximately 0.6 Å from this axis to contact the wall of the 4.5-Å wide void at a point opposite the O(6) atoms of glucoses 1 and 5, Figure 5. These two factors apparently compensate for the relatively unfavorable glucosidic bond conformation and suggest that a change to a more favorable macrocyclic conformation can account for some or all of the free-energy change accompanying the inclusion process.

The combined effect of the individual rotations about the glucosidic linkage is responsible for the overall conical shape of the cyclodextrin ring. The α -CD molecule is tapered with the O(2)–O(3) side the larger, more open side. The atoms C(1) and C(4) are not in the mean plane of the very nearly coplanar O(4) atoms (Table VI) but rather are displaced by up to 0.836 Å toward the O(6) side of the α -CD ring.

The overall macrocyclic conformation of the cyclodextrin ring with (H₂O)₂ as the included substrate is distinctly different from that found in the case of the crystallographically nonisomorphous potassium acetate inclusion complex. With potassium acetate as the included substance, a twofold crystallographic axis was found to coincide with α -CD torus axis. The individual α -CD molecules were found related to one another in a highly symmetrical manner with one cyclodextrin positioned directly over another, O(2)–O(3) side against the O(6) side. In the case of the potassium acetate inclusion complex the cyclodextrin ring is tapered to a much lesser extent than in the (H₂O)₂ inclusion complex and the diameter of the circle of O(2)–O(3) atoms is reduced such that the formation of continuous O(2)···O(3) hydrogen bonds between adjacent glucose residues is possible. With α -CD hexahydrate there is only one O(2)···O(3) distance less than 2.9 Å and the formation of a continuous ring of hydrogen bonds is excluded.

Hydrogen Bonding. With each hydrogen atom attached to oxygen potentially involved in hydrogen bonding, 30 hydrogen bonds are possible for each formula (C₆H₁₀O₅)₆·6H₂O. Omitting momentarily the four potential hydrogen bonds associated with the two

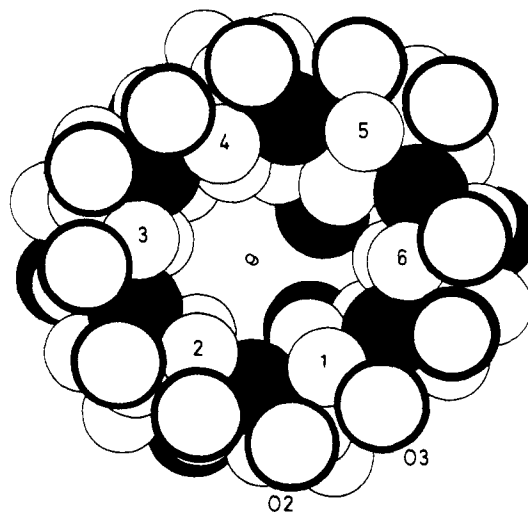


Figure 5. α -Cyclodextrin molecule in the hexahydrate complex, projected onto the mean plane of the O(4) atoms. O(4) and O(6) atoms are shown as solid circles; O(2) and O(3) atoms are designated by thicker lines; carbon and hydrogen atoms are represented by thin lines and hydrogen atoms bound to atoms O(2), O(3), and O(6) have been omitted. The glucose residues are numbered on the hydrogens bound to C(N3).

water molecules included in the center of the cyclodextrin ring, only six of the remaining 26 possible hydrogen bonds could not be definitely assigned, including the assignment of direction, when the locations of the hydrogen atoms estimated from a difference Fourier synthesis were taken into consideration. The hydrogen bonds are listed in Table VIII and are shown in Figure 6.

(a) Water Inclusion. Water molecules A and B are located within the α -cyclodextrin void, Figure 6. From the results of this study it is clear that water A is involved in hydrogen bonding to O(56) (2.740 Å) with, from the hydrogen positions, water as the donor. The existence of other hydrogen bonds involving the included water molecules is less clear. Both O(16) and O(56) hydroxyl groups are in a trans position relative to C(14) and C(54). The water A to O(16) distance is 2.945 Å, O(16) participates as a donor to O(36) from another cyclodextrin molecule (2.868 Å), and the O(56)–water A–O(16) angle is 78.2°. These observations suggest that water A is probably not simultaneously hydrogen bonded to both O(56) and O(16). Water B is located 2.931 Å from water A, and water A is in satisfactory position to act as a donor to both O(56) and water B. The O(56)–water A–water B

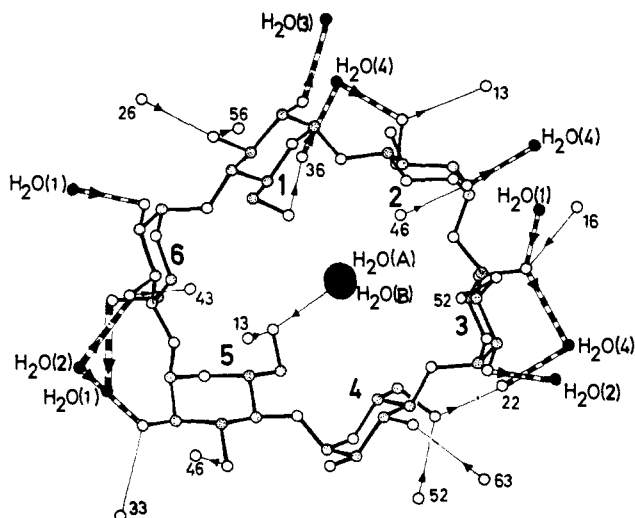


Figure 6. Hydrogen bonding scheme. Hydrogen bonds shown striped appear in Figure 7. Water oxygen atoms are represented by solid circles, other oxygen atoms by open circles, and carbon atoms by shaded circles.

Table VIII. Hydrogen Bonds of α -CD Hexahydrate^a

Donor	Acceptor	O-O distance	O-H-O angle
O(12)	Water 3	2.809	111
O(13)	O(56)	2.603	119
O(16)	O(36)	2.817	158
O(22)	Water 4	2.696	175
O(23)	<i>b</i>		
O(26)	O(13)	2.807	140
O(32)	Water 2	2.718	157
O(33)	O(52)	2.879	78
O(36)	Water 4	2.874	117
O(42)	<i>c</i>		
O(43)	<i>d</i>		
O(46)	O(22)	2.836	175
O(52)	<i>e</i>		
O(53)	O(46)	2.688	45
O(56)	<i>f</i>		
O(62)	<i>g</i>		
O(63)	O(43)	2.783	133
O(66)	Water 1	2.888	43
Water 1	Water 2	2.854	165
Water 1'	O(62)	2.888	53
Water 2	O(63)	2.764	155
Water 2'	O(52)	2.711	73
Water 3	O(36)	2.823	166
Water 3'			
Water 4	Water 1	2.878	60
Water 4'	O(26)	2.817	78
Water A	O(56)	2.740	165
Water A'	Water B	2.931	130
Water B			
Water B'			

^a All the O...O distances less than 3.0 Å have been listed. Hydrogen bonds with O-H...O angles $\ll 90^\circ$ are questionable.

^b The only possibility for hydrogen bonding is intramolecular to atom O(12). The O(12)-O(23) distance is 2.941 Å. ^c Possible hydrogen bond to included water molecule B (2.971 Å) or intramolecular bonding to O(43), 2.880 Å, or to O(54), 2.776 Å. ^d Acceptor from O(36); possible intramolecular hydrogen bond to O(32), 2.823 Å. ^e Hydrogen bonded to O(33), direction assigned arbitrarily. Also an acceptor from water 2. ^f Acceptor from O(13) and water 2. ^g Acceptor from water 1.

angle is 110.2° . The possibility of a 2.971-Å hydrogen bond between water B and O(42) leads to a water A-water B-O(42) angle of 141.3° . The water A-O(56)

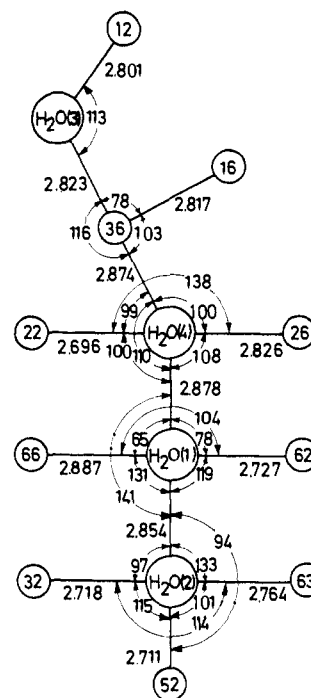


Figure 7. Nonincluded water hydrogen bonding.

hydrogen bond is the only contact between the included water molecule and the cyclodextrin less than 2.9 Å.

(b) **Intermolecular Hydrogen Bonding.** The cyclodextrin hydroxyl groups are principally involved in intermolecular hydrogen bonding or in hydrogen bonding to water. With only two exceptions all O(2), O(3), and O(6) hydroxyl groups are involved in at least one such hydrogen bond, Figure 6, Table VIII. Only O(23) cannot participate in intermolecular hydrogen bonding, and such a role for O(42) is also unlikely.

Hydroxyl group O(42) is perhaps involved as a donor in weak intramolecular hydrogen bonding to the included water molecule B or in intramolecular bonding to the bridging oxygen O(54) (2.776 Å) or to the O(3) hydroxyl group of the same glucopyranose O(43), 2.897 Å.

The three remaining hydrogen atoms for which no definite hydrogen bonding could be assigned are constituents of water molecules 1, 2, 3, and 4. The utilization of the hydrogen bonding potential of the water of crystallization is nonetheless very high. The four water molecules form a network with one another and the cyclodextrin, Figure 7. The coordination around each of the nonincluded water molecules is approximately tetrahedral.

Intermolecular Packing. The spatial relationship between individual α -CD molecules is shown in Figure 8. Alternating vertical columns of molecules are displaced relative to one another by $a/2$ which is equivalent to approximately one-half of the diameter of the α -CD molecule. In Figure 8 the larger "top" diameter is the circle formed by joining adjacent O(2) and O(3) atoms. The smaller "bottom" plane is the circle in the center of each molecule that is formed by joining successive O(4) atoms. Where visible, O(56) is shown covalently bonded to C(56) and hydrogen bonded to one of the two included water molecules.

The structure is of the cage type with the voids in

the centers of adjacent cyclodextrin molecules not aligned to form continuous channels; the O(6) side of each α -CD molecule is occluded by the O(6) side of one contiguous α -CD molecule and the O(2), O(3) side of another α -CD molecule. The O(2), O(3) side of each α -CD is also occluded by two other α -CD molecules, one related by a c translation and the other related by a screw dyad axis parallel to c .

Conclusion

The structure of α -CD hexahydrate, the $(\text{H}_2\text{O})_2$ inclusion complex, is of particular interest insofar as it represents the "empty" α -cyclodextrin molecule occurring in aqueous solution before adduct formation takes place. As has been found in this diffraction study, the macrocyclic conformation of the cyclodextrin ring and the orientations of the O(6) hydroxyl groups are different when $(\text{H}_2\text{O})_2$ is the included substance from that observed with other guest molecules such as 1-propanol, methanol, potassium acetate, and I_2 .

When α -CD hexahydrate is compared with the potassium acetate adduct, the observed differences in cyclodextrin conformation are attributable not only to the nature of the included substance but also to the influences of the crystalline environment. However, when α -CD hexahydrate is compared to the I_2 adduct,⁵ the observed differences in conformation must represent primarily the influence of the guest molecule as the two crystal structures are substantially isomorphous. In the I_2 inclusion complex all O(6) hydroxyl groups are in the gauche, gauche conformation directed away from the center of the cyclodextrin molecule. Furthermore, the irregularity of the glucosidic bond conformation found in the hexahydrate structure is not present; the molecule is more nearly hexagonal.

The observation of two distinctly different conformations reflecting the influence of the substrate molecule suggests a possible mechanism for adduct formation. (1) In aqueous solution α -CD exists in a conformation similar to that found in this solid-state hexahydrate structure. (2) In this conformation the α -CD molecule is in a "strained" high-energy conformation which is compensated by the formation of hydrogen bonds to the included water and by the reduction of the otherwise "empty" volume in the cyclodextrin void. (3) Thermally or under the influence of the prospective guest molecule CD undergoes a conformational change with the gauche, trans O(6) hydroxyl groups rotating to the gauche, gauche form necessary for the end product. (4) The guest molecule displaces the two included water molecules and assumes the spectrum of accessible orientations directed

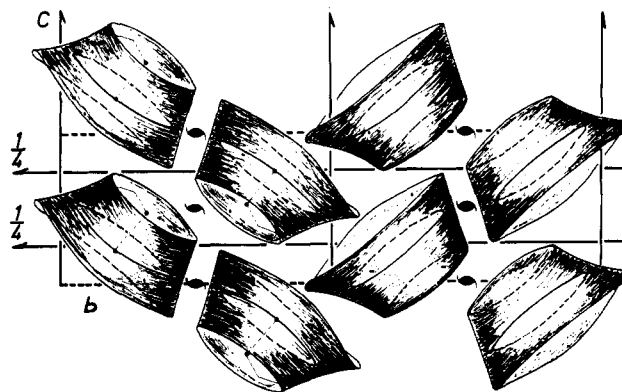


Figure 8. α -CD $(\text{H}_2\text{O})_6$ intermolecular packing scheme.

by the available thermal energy and the nature of the interactions between guest molecule and cyclodextrin, including the possible formation of hydrogen bonds.

Compared with the mechanism of inclusion formation of α -CD proposed earlier on the basis of kinetic and thermodynamic data,²⁰ the described mechanism gives further details as to the interactions between the α -CD and substrate molecules. As now a conformational change is involved in complex formation, the mechanism is similar to the "induced fit" mechanism proposed for the interaction of enzymic proteins with their cofactors and substrates²¹ and suggests that α -CD is an even better enzyme model than previously assumed.^{22,23}

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Supplementary Material Available. A listing of structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 \times 148 mm, 24 \times reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for \$3.00 for photocopy or \$2.00 for microfiche, referring to code number JACS-74-3630.

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