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STRUCTURE OF INCLUSION COMPLEXES OF CYCLOMALTOHEPTAOSE (CYCLOHEPTAAMYLOSE): CRYSTAL STRUCTURE OF THE BENZOCAINE ADDUCT

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ABSTRACT

Cyclomaltoheptaose (cycloheptaamylose) has been crystallized with benzocaine (ethyl *p*-amino-benzoate) as the guest molecule. The complex crystallizes in space group C2, with unit-cell dimensions *a* 1.8746(9), *b* = 2.4528(4), and *c* = 1.5658(5) nm, and $\beta = 110.21°$(3). The crystal structure was solved by using the phases from a previously determined, isomorphous crystal-structure. The cyclomaltoheptaose exists as a dimer in the crystal by means of extensive hydrogen-bonding across the secondary-hydroxyl ends of two cyclomaltoheptaose molecules. A continuous channel throughout the crystal is achieved by stacking of these dimer units. The two halves of the cyclomaltoheptaose dimer are related by the crystallographic, two-fold axis, but the benzocaine guests in each half do not follow this symmetry, and are statistically disordered. The ester groups are at the secondary-hydroxyl end of the cyclomaltoheptaose, and the guests are disordered (to avoid overcrowding around the crystallographic, two-fold axis). A lone, water molecule is trapped in the cavity at the secondary hydroxyl interface, and forms a link between the ester groups of the two guest molecules by hydrogen bonding to the carbonyl oxygen atom of both. The *p*-amino group of each guest in the dimer pair has a different, and independent, hydrogen-bonding scheme. One amino group hydrogen-bonds directly to a primary hydroxyl group of the cyclomaltoheptaose (in the *gauche-trans* position), whereas the other is hydrogen-bonded *via* a water molecule in the cavity at the primary-hydroxyl end to a different primary hydroxyl group.

INTRODUCTION

The cyclomaltaoses (cycloamyloses, cyclodextrins) are cyclic oligosaccharides containing 6 to 12 D-glucopyranosyl residues linked α-(1→4). They have attracted great attention as models for such enzymes as the serine acylase enzymes, in particular, chymotrypsin[1,2]. Cyclomaltohexaose (cyclohexaamylose, α-cyclodextrin), cyclomaltoheptaose (cycloheptaamylose, β-cyclodextrin), and, to a lesser extent, cyclomalto-octaose (cyclooctaamylose, γ-cyclodextrin) have been studied, in order to examine the mode of binding of organic molecules in their cavities. This complexation

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process is the basis for all of the wide variety of phenomena produced by the cyclomaltaoses, including catalysis of ester hydrolysis. These phenomena, and other applications of the cyclomaltaoses in research and industry, have recently been reviewed by Saenger[3].

Early studies showed that the cyclomaltaoses cause stereoselective acceleration of the cleavage of phenyl esters[1,4]. Cyclomaltoheptaose was a better catalyst than cyclomaltohexaose. Benzocaine (ethyl *p*-aminobenzoate) has been examined as a possible substrate for ester hydrolysis. However, it was found that the alkaline hydrolysis of such alkyl esters as methyl or ethyl benzoate and ethyl *trans*-cinnamate is retarded or totally inhibited by cyclomaltoheptaose[4-6]. Hydrolysis of benzocaine was also inhibited by cyclomaltohexaose. Lach and Chin[6] proposed that the unreactivity of the complexed alkyl esters could be due to nonproductive binding, in which the carbonyl carbon atoms of the alkyl esters were located at some distance from the secondary hydroxyl groups in the cavity of the cyclomaltaose. They also suggested that, when in the cavity, the carbonyl carbon atoms would be protected from attack by the hydroxide ion, and supported this proposal by the observation that the hydrolysis of atropine[7], ethyl *o*-aminobenzoate, and ethyl *m*-aminobenzoate, which cannot be totally included in the cyclomaltohexaose cavity because of steric hindrance, is slightly accelerated by it[5].

Alternatively, Bender and co-workers[4] proposed that the unreactivity of complexed alkyl esters could be attributed to an unfavorable partitioning of a tetrahedral intermediate. Attack by the alkoxide ion of the cyclomaltaose on the carbonyl carbon atom of the substrate would lead to formation of a tetrahedral intermediate having both the cyclomaltaose alkoxide ion and the alkoxide ion derived from the alkyl ester as potential leaving-groups. The tetrahedral intermediate would preferentially revert to the reactants, as the cyclomaltaose alkoxide ion is a better leaving-group (lower pKa) than the alkoxide ion derived from the alkyl ester.

This X-ray crystal-structure determination was undertaken in order to examine the binding of benzocaine in the cavity of cyclomaltoheptaose and attempt to relate the results to the observed unreactivity of the alkyl esters. Owing to the pharmacological importance of benzocaine, the results would also be significant in the area of drug stabilization and solubilization. In view of the prerequisite of binding of guests inside the cavity for all phenomena catalyzed by cyclomaltaoses, any detailed information on this binding is important.

EXPERIMENTAL

Crystals of the complex of cyclomaltoheptaose with benzocaine were prepared as previously described[8]. The crystals are monoclinic, space group C2, with unit-cell dimensions a = 1.8746(9), b = 2.4528(4), and c = 1.5658(6) nm, and β = 110.21°(3). The crystals are isomorphous with those of cyclomaltoheptaose complexed with a series of substituted benzoic acids and phenols; the structural studies on these, and the method of solution of the crystal structure, have been described previously[9].

The phases determined in that study were used to solve the present, isomorphous, crystal structure.

Three-dimensional, X-ray diffraction data were collected at the University of Leeds, England, on a Nonius, automated, four-circle diffractometer, using CuKα radiation. As the crystals become opaque through loss of water, they were sealed in quartz capillary-tubes during data collection at room temperature. There were 3,058 observed reflections (those with Io > σIo) in the range $3° \leqslant \theta \leqslant 50°$.

STRUCTURE DETERMINATION AND REFINEMENT

Atomic coordinates for cyclomaltoheptaose (omitting the primary hydroxyl groups) from the isomorphous crystal-structures previously determined[9] were subjected to several cycles of least-squares refinement. The difference map clearly showed the water molecules and primary hydroxyl groups. Further refinement, including the primary hydroxyl groups and water molecules, initially showed a rather incomprehensible distribution of electron density in the cavity of the cyclomaltoheptaose, and chemical sense was not obvious in this first map. A sharpened, Fourier synthesis was calculated over the cavity region, using Eo as the amplitude, instead of Fo. This sharpened map showed the outline of a planar ring. Attempts were made to fit a model of benzocaine to this map, and, during this process, it was realized that there were two intersecting benzene rings, each with a well-resolved nitrogen peak and carboxyl group. At this point, it was obvious that the benzocaine was statistically disordered, each cyclomaltoheptaose moiety of the dimer enclosing a benzocaine molecule that did not conform to the crystallographic, two-fold axis. Atomic positions were taken from the original, difference-Fourier synthesis, and each benzocaine molecule was assigned half-weight. However, least-squares refinement of these positions was unsuccessful, particularly for the ring atoms, and the guest molecules were refined solely by difference-Fourier synthesis. Two half-weight, water molecules associated with the benzocaine molecules were found, one at the primary end, and one at the secondary end, enclosed in the cavity by dimer formation.

The cyclomaltoheptaose molecules, and the water molecules external to the cavity, were refined by the least-squares method. The quantity minimized in the least-squares refinement was $\Sigma\omega(|Fo| - |Fc|)^2$, where $\omega = 1/\sigma(Fo)$. The atomic scattering-factors were taken from the literature[10]. The X-Ray 76 system of computer programs was used for most calculations. The final R index was 0.16 for 3,058 observed reflections.

RESULTS AND DISCUSSION

The atomic coordinates and temperature parameters are given in Table I. The numbering scheme is shown in Fig. 1. The corresponding bond lengths and angles are listed in Table II. The dihedral angles of the cyclomaltoheptaose molecule are given in Table III. Tables IV and V list the intra- and inter-molecular, hydrogen-

TABLE I

FRACTIONAL COORDINATES AND THERMAL PARAMETERS OF NON-HYDROGEN ATOMS[a]

Molecule	*Atom*	*x*	*y*	*z*	*U*
Cyclomaltoheptaose					
	C-11	7218(16)	1759(12)	3178(19)	4.0(8)
	C-12	6955(16)	2019(12)	3894(19)	4.3(8)
	C-13	6138(15)	1892(11)	3777(18)	3.6(7)
	C-14	5673(15)	2133(11)	2825(18)	3.2(7)
	C-15	5946(15)	1853(11)	2093(18)	3.5(7)
	C-16	5534(17)	2133(13)	1124(20)	5.1(8)
	O-12	7459(10)	1833(8)	4778(12)	4.4(5)
	O-13	5921(10)	2208(8)	4438(12)	4.3(5)
	O-14	4898(10)	1981(7)	2641(12)	3.9(5)
	O-15	6710(9)	1950(7)	2300(11)	3.7(5)
	O-16	5677(11)	2705(9)	1145(13)	5.6(6)
	C-21	4341(17)	2444(13)	2308(21)	5.1(9)
	C-22	3685(17)	2460(13)	2972(21)	5.1(8)
	C-23	3506(16)	1919(13)	3020(20)	4.6(8)
	C-24	2822(16)	1860(12)	2060(19)	4.3(8)
	C-25	3391(16)	1774(12)	1425(20)	4.5(8)
	C-26	2857(19)	1748(15)	408(24)	6.8(10)
	O-22	4469(11)	2572(9)	3923(14)	5.8(6)
	O-23	3076(10)	1993(8)	3651(12)	4.8(5)
	O-24	2528(10)	1365(7)	2073(12)	3.9(5)
	O-25	3827(10)	2291(8)	1474(13)	4.7(5)
	O-26	2363(14)	2182(10)	163(16)	8.1(7)
	C-31	1754(16)	1388(12)	1725(19)	4.7(8)
	C-32	1378(17)	1168(13)	2443(21)	5.1(9)
	C-33	1724(15)	559(11)	2676(18)	3.6(7)
	C-34	1404(15)	227(11)	1804(18)	3.7(7)
	C-35	1686(17)	464(13)	1044(20)	5.2(9)
	C-36	1073(23)	193(17)	44(26)	8.6(12)
	O-32	1650(11)	1480(9)	3214(14)	5.8(6)
	O-33	1335(11)	350(9)	3286(13)	5.9(6)
	O-34	1783(9)	−304(8)	2056(12)	4.0(5)
	O-35	1423(11)	1040(8)	895(13)	5.2(6)
	O-36	1490(16)	345(12)	−526(19)	10.9(9)
	C-41	1271(19)	−784(14)	1813(23)	6.4(10)
	C-42	1548(14)	−1132(10)	2648(17)	3.0(7)
	C-43	2365(15)	−1282(11)	2972(18)	3.3(7)
	C-44	2487(16)	−1615(12)	2159(19)	4.3(8)
	C-45	2223(16)	−1216(12)	1329(20)	4.4(8)
	C-46	2238(17)	−1569(13)	435(21)	5.4(9)
	O-42	1396(11)	−838(8)	3415(13)	5.4(6)
	O-43	2548(10)	−1678(8)	3715(12)	4.6(5)
	O-44	3246(9)	−1739(7)	2387(11)	3.5(4)
	O-45	1449(11)	−1076(8)	1112(14)	5.3(6)
	O-46	1768(11)	−2007(9)	288(14)	5.9(6)
	C-51	3423(16)	−2293(13)	2282(20)	5.0(8)
	C-52	3984(17)	−2497(13)	3127(21)	5.4(9)
	C-53	4726(16)	−2161(12)	3398(19)	3.6(7)
	C-54	5046(14)	−2216(11)	2641(17)	3.3(7)
	C-55	4437(15)	−2015(12)	1752(18)	4.0(7)
	C-56	4702(19)	−2111(14)	886(22)	6.2(9)

TABLE I *(continued)*

Molecule	*Atom*	*x*	*y*	*z*	*U*
	O-52	3670(9)	−2448(8)	3864(12)	4.1(5)
	O-53	5260(10)	−2367(8)	4230(12)	4.6(5)
	O-54	5692(9)	−1865(7)	2832(11)	3.4(5)
	O-55	3771(9)	−2342(8)	1586(12)	4.4(5)
	O-56	4889(12)	−2631(9)	852(14)	6.2(6)
	C-61	6341(15)	−2103(12)	2829(18)	4.0(8)
	C-62	7002(13)	−1990(10)	3752(16)	2.7(6)
	C-63	7095(17)	−1377(13)	3933(21)	4.6(8)
	C-64	7364(15)	−1141(11)	3151(17)	3.1(7)
	C-65	6729(15)	−1272(12)	2211(18)	3.6(7)
	C-66	6977(20)	−1170(15)	1335(24)	7.1(10)
	O-62	6779(9)	−2240(7)	4486(12)	4.1(5)
	O-63	7724(10)	−1286(8)	4763(12)	4.5(5)
	O-64	7392(10)	−577(8)	3248(13)	4.3(5)
	O-65	6596(10)	−1877(8)	2132(12)	4.4(5)
	O-66	6879(37)	−688(28)	1067(44)	12.6(23)
	O-66′	7711(27)	−1392(19)	1526(32)	7.6(14)[b]
	O-66″	6376(36)	−1219(27)	498(43)	12.6(22)
	C-71	8127(17)	−323(13)	3285(20)	5.0(8)
	C-72	8450(18)	−6(14)	4148(21)	5.8(9)
	C-73	7793(15)	459(11)	4104(18)	3.7(7)
	C-74	7708(16)	831(11)	3283(19)	4.0(7)
	C-75	7387(17)	434(13)	2416(20)	4.9(8)
	C-76	7384(18)	797(13)	1561(21)	5.8(9)
	O-72	8550(11)	−305(9)	4939(14)	5.7(6)
	O-73	8109(12)	817(9)	4888(14)	6.0(6)
	O-74	7077(10)	1169(7)	3232(12)	4.2(5)
	O-75	7968(9)	25(8)	2482(11)	4.1(5)
	O-76	7128(14)	448(11)	745(17)	8.6(8)
Benzocaine					
	C-101	4678[c]	−200	2740	23(5)
	C-102	3929	−143	2188	16(5)
	C-103	3937	37	1424	7(2)
	C-104	4569	259	1285	9(3)
	C-105	5345	204	1910	23(6)
	C-106	5395	−83	2700	11(3)
	C-107	4626	−474	3492	39(9)
	N-108	4644	482	486	23(4)
	O-109	5226	−668	4079	16(3)
	O-110	4310	−668	4021	18(3)
	C-111	4012	−333	4479	23(6)
	C-112	3190	−204	4597	16(4)
	C-201	5402	184	7502	25(4)
	C-202	5962	−212	7887	28(7)
	C-203	5903	−387	8581	15(4)
	C-204	5174	−237	8636	21(5)
	C-205	4584	156	8260	28(7)
	C-206	4720	389	7550	11(3)
	C-207	5521	489	6709	52(11)
	N-208	5130	−500	9436	18(4)
	O-209	6028	463	6390	24(4)
	O-210	5228	734	5911	15(2)
	C-211	5000	941	5000	8(3)
	C-212	5000	1604	5000	19(8)

TABLE I *(continued)*

Molecule	*Atom*	*x*	*y*	*z*	*U*
Water					
	W-1	4586(13)	3541(9)	795(16)	8(1)
	W-2	4147(28)	3341(22)	4874(33)	21(3)
	W-3	539(21)	1459(17)	4263(25)	17(1)
	W-4	9681(31)	45(26)	2271(36)	22(3)
	W-5	9079(18)	1467(14)	1064(22)	13(1)
	W-6	−624(19)	1094(14)	2727(24)	14(1)
	W-7	5477(25)	3515(19)	3966(30)	18(2)
	W-8	5601(62)	973(50)	−264(80)	19(5)[d]
	W-9	5943(25)	333(19)	4375(30)	18(3)
	W-10	9135(30)	−723(23)	1763(36)	20(3)

[a]Positional parameters are multiplied by 10^4; thermal parameters by 100. The numbering of atoms is defined as A(m,n) where A is the atom type, m is the residue number, and n is the atom number within a residue (see also, Fig. 1). W denotes a water molecule. The benzocaine molecules have m values of 10 and 20, respectively. [b]These three atomic positions are disordered positions for the primary-hydroxyl oxygen atom attached to C-56. They were assigned population parameters of 0.33 in the structure-factor calculations. [c]The fractional coordinates of the benzocaine molecules were not refined by the least-squares method, and no standard deviations are given. The population parameters of all of the atoms in both of the benzocaine molecules were restricted to 0.50. [d]The population parameters of W-8 and W-9 were restricted to 0.5. The other water molecules were full-weight, except for W-7 and W-10, which have a weight of 0.80.

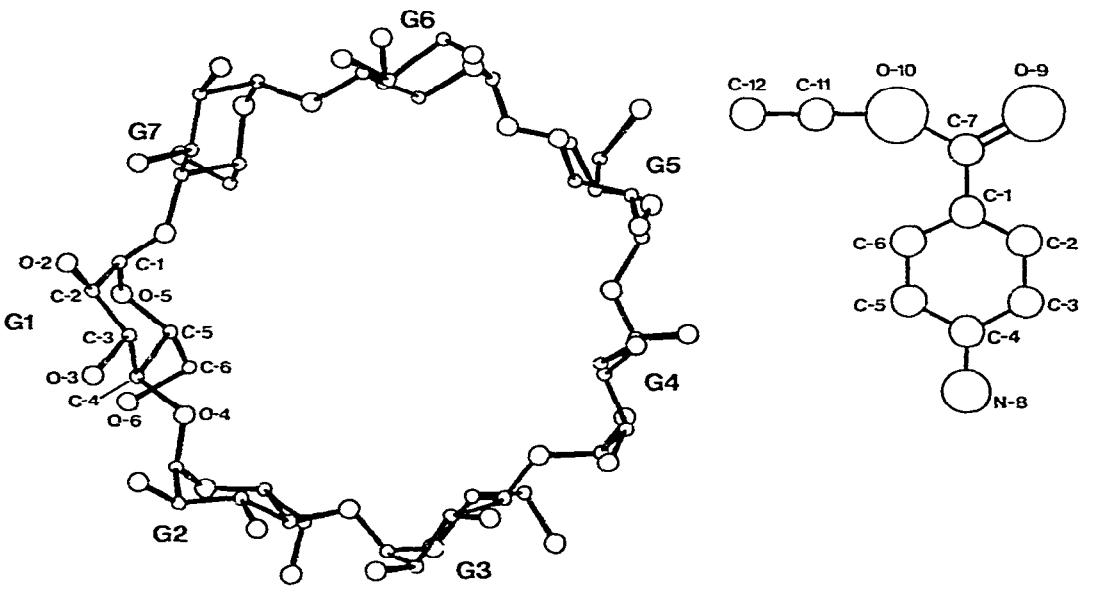


Fig. 1. Numbering scheme for the cyclomaltoheptaose and benzocaine molecules.

TABLE II

BOND LENGTHS (pm) AND ANGLES (DEGREES) OF THE CYCLOMALTOHEPTAOSE MOLECULE

Atoms	*G1*	*G2*	*G3*	*G4*	*G5*	*G6*	*G7*
Cyclomaltoheptaose							
Bond lengths							
C-1–C-2	151(5)	156(6)	161(5)	150(4)	147(5)	157(5)	150(5)
C-1–O-4′[a]	145(4)	151(4)	136(4)	148(4)	142(4)	135(4)	150(4)
C-2–C-3	151(4)	152(5)	162(4)	149(4)	154(4)	153(4)	166(4)
C-2–O-2	145(4)	154(5)	137(4)	151(4)	147(5)	149(4)	140(4)
C-3–C-4	156(4)	153(5)	152(4)	159(4)	151(5)	159(5)	154(4)
C-3–O-3	146(4)	149(4)	148(4)	146(3)	143(4)	144(4)	146(4)
C-4–C-5	157(5)	155(5)	157(5)	157(4)	155(5)	157(5)	161(4)
C-4–O-4	143(3)	143(4)	147(3)	138(4)	143(3)	139(3)	142(4)
C-5–O-5	137(3)	150(4)	149(4)	141(4)	143(3)	150(3)	146(4)
C-5–C-6	160(4)	156(5)	172(5)	165(5)	162(5)	161(5)	161(5)
C-6–O-6	143(4)	138(4)	143(6)	136(4)	133(4)	125(8)[b]	147(4)
C-1–O-5[b]	148(3)	138(4)	150(4)	144(5)	146(4)	145(4)	146(4)
Bond angles							
C-1–C-2–C-3	114(2)	113(3)	103(3)	116(3)	111(3)	110(2)	105(2)
C-1–C-2–O-2	109(4)	106(2)	108(2)	109(2)	109(3)	107(2)	115(3)
O-2–C-2–C-3	111(3)	107(2)	109(2)	108(2)	108(2)	108(2)	106(3)
C-2–C-3–C-4	104(2)	103(2)	107(2)	106(2)	108(2)	105(2)	108(3)
C-2–C-3–O-3	109(2)	107(2)	103(2)	112(2)	110(2)	109(2)	107(2)
O-3–C-3–C-4	106(2)	107(2)	105(2)	104(2)	110(2)	105(2)	104(2)
C-3–C-4–C-5	108(2)	106(2)	111(2)	105(2)	108(2)	108(2)	104(2)
C-3–C-4–O-4	107(2)	105(2)	103(2)	109(2)	109(2)	107(2)	104(3)
O-4–C-4–C-5	108(2)	108(2)	106(2)	111(3)	108(2)	107(2)	104(2)
C-4–C-5–O-5	109(2)	106(2)	107(3)	111(3)	108(2)	109(2)	108(2)
C-4–C-5–C-6	109(2)	111(2)	105(3)	106(2)	111(2)	115(2)	105(2)
O-5–C-5–C-6	106(2)	103(2)	98(2)	104(2)	106(2)	100(2)	103(3)
C-5–C-6–O-6	112(2)	113(3)	98(3)	111(3)	110(3)	112(5)[c]	108(3)
C-5–O-5–C-1	117(2)	118(2)	113(2)	115(2)	114(2)	114(2)	113(2)
O-5–C-1–C-2	107(2)	106(2)	105(2)	106(3)	106(3)	105(2)	118(3)
C-1′–O-4–C-4	114(2)	116(2)	115(2)	116(2)	116(2)	115(3)	114(2)
C-2–C-1–O-4′	105(2)	105(2)	112(2)	103(2)	110(2)	110(2)	109(3)
O-5–C-1–O-4′	107(2)	107(2)	113(3)	107(3)	110(2)	112(2)	107(2)

Benzocaine					
Bond lengths			**Bond angles**		
Bond	*Mol. 1*	*Mol. 2*	*Angle*	*Mol. 1*	*Mol. 2*
C-1–C-2	138	140	C-2–C-1–C-6	138	142
C-1–C-6	140	140	C-2–C-1–C-7	103	113
C-1–C-7	139	153	C-6–C-1–C-7	119	105
C-2–C-3	128	121	C-1–C-2–C-3	107	110
C-3–C-4	139	145	C-2–C-3–C-4	125	110
C-4–C-5	145	143	C-3–C-4–C-5	125	139
C-4–N-8	142	144	C-3–C-4–N-8	130	106
C-5–C-6	140	135	C-5–C-4–N-8	105	113
C-7–O-9	127	122	C-4–C-5–C-6	113	107
C-7–O-10	127	132	C-5–C-6–C-1	112	109
O-10–C-11	133	143	C-1–C-7–O-9	119	131
C-11–C-12	165	163	C-1–C-7–O-10	157	149
			O-9–C-7–O-10	83	76
			O-10–C-11–C-12	140	111

[a]The primed numbers refer to atoms on neighboring residues. [b]The bond lengths from C-6 to the equal-weight, disordered positions of O-6 on residue G6 are 141(6) and 141(7) pm. [c]The bond angles C-5–C-6–O-6 for the disordered, primary hydroxyl group on residue G6 are 108(3) and 114(4)°.

TABLE III

DIHEDRAL ANGLES OF THE CYCLOMALTOHEPTAOSE MOLECULE

Dihedral angle (degrees)	*G1*	*G2*	*G3*	*G4*	*G5*	*G6*	*G7*
C-1–C-2–C-3–C-4	−61	−65	−66	−60	−60	−66	−59
C-2–C-3–C-4–C-5	60	67	63	58	58	62	61
C-3–C-4–C-5–O-5	−59	−64	−59	−59	−62	−58	−60
C-4–C-5–O-5–C-1	59	62	64	62	66	61	60
C-5–O-5–C-1–C-2	−57	−55	−70	−57	−65	−63	−62
O-5–C-1–C-2–C-3	57	57	67	58	59	65	58
C-4–C-5–C-6–O-6	58	52	−150	57	53	−92[a]	176
O-5–C-5–C-6–O-6	−60	−62	80	−59	−63	152	61
O-2–C-2–C-3–O-3	66	64	69	63	57	67	65
O-2–C-2–C-3–C-4	179	178	179	176	177	177	177
O-2–C-2–C-1–O-5	179	173	−178	−179	−179	−179	178
O-3–C-3–C-4–O-4	−69	−64	−77	−65	−67	−72	−71
O-3–C-3–C-4–C-5	175	180	171	177	179	175	178
O-3–C-3–C-2–C-1	−174	−178	−176	−173	180	−177	−171
O-2–C-2–C-1–O-4′[b]	67	58	57	65	60	59	57
O-5–C-1–O-4′–C-4′	117	121	115	109	115	118	115
C-2–C-1–O-4′–C-4′	−130	−125	−126	−134	−127	−125	−121
C-1′–O-4–C-4–C-3	134	133	132	129	129	128	128
C-1′–O-4–C-4–C-5	−110	−115	−115	−116	−117	−117	−121

[a]The additional values of C-4–C-5–C-6–O-6 and O-5–C-5–C-6–O-6 for the disordered positions of the primary-hydroxyl oxygen atom on G6 are +43, −150°, and −73, 74°, respectively. [b]The primed numbers refer to atoms in neighboring residues.

bond data. A diagram showing the hydrogen bonding and close contacts of the cyclomaltoheptaose dimer is given in Fig. 2a, and a diagram of the hydrogen bonding involving the guests, in Fig. 2b. The seven D-glucosyl residues are in the $^4C_1$(D) conformation. Four of the primary hydroxyl groups point away from the cavity, and are in the *gauche-gauche* orientation. Two other primary hydroxyl groups point in, towards the cavity, and are in the *gauche-trans* orientation. The seventh hydroxyl group is disordered, and appears in three positions: one pointing away from the cavity, and two pointing towards the cavity (see Figs. 3 and 4). This is in contrast to the isomorphous complexes with substituted phenols and benzoic acids[9], where all of the primary hydroxyl groups point away from the cavity in the *gauche-gauche* orientation. In the present crystal structure, the twist of the hydroxyl groups in order to point into the cavity allows hydrogen bonding to the *p*-amino group of the benzocaine guest and to a water molecule in the cavity at the primary-hydroxyl end of the cyclomaltoheptaose (see Figs. 3 and 4). This hydrogen-bonding scheme will be discussed in more detail later.

The cyclomaltoheptaose molecules form a head-to-head dimer by means of strong hydrogen-bonds across the secondary-hydroxyl ends of the cyclomaltoheptaose (see Fig. 2a). The dimers then stack together as in a cylinder. The crystallographic,

TABLE IV

INTRAMOLECULAR HYDROGEN-BOND LENGTHS AND ANGLES

Hydrogen-bond	*Distance (pm)*	*Hydrogen-bond angle*	*Angle (degrees)*
Cyclomaltoheptaose			
O-12···O-73	275(3)	C-12–O-12···O-73	118(2)
O-22···O-13	271(4)	C-22–O-22···O-13	122(2)
O-32···O-23	282(3)	C-32–O-32···O-23	120(2)
O-42···O-33	292(3)	C-42–O-42···O-33	116(2)
O-52···O-43	278(3)	C-52–O-52···O-43	119(2)
O-62···O-53	275(3)	C-62–O-62···O-53	119(1)
O-72···O-63	282(3)	C-72–O-72···O-63	118(2)
		C-13–O-13···O-22	118(1)
		C-23–C-23···O-32	118(2)
		C-33–O-33···O-42	112(2)
		C-43–O-43···O-52	119(2)
		C-53–O-53···O-62	117(2)
		C-63–O-63···O-72	118(2)
		C-73–O-73···O-12	117(1)
Benzocaine			
N-108···W-8	274	C-104–N-108···W-8	146
N-108···O-76 I[a]	321	C-104–N-108···O-76 I[a]	97
O-109···W-10	276	C-107–O-109···W-10	93
N-208···O-66″ II	292	C-201–N-208···O-66″ II	120
N-208···O-66 III	362	C-201–N-208···O-66 III	105
O-209···W-10	312	C-207–O-209···W-10	130

[a]The Roman numerals refer to the following equivalent positions: I, $-x+1, y, -z$; II, $x, y, 1+z$; III, $-x+1, y, -z+1$.

two-fold axis runs perpendicular to the seven-fold axis through the head-to-head and tail-to-tail connections. The secondary-hydroxyl ends of two cyclomaltoheptaose molecules are directly hydrogen-bonded together. The primary ends are farther apart, and are connected by hydrogen-bonded, water molecules. The parallel columns of cyclomaltoheptaose are connected by water molecules, which form narrower columns, parallel to them. Within the cyclomaltoheptaose column is a continuous channel wherein the guests are found. This overall structure is similar to that previously described[9], except that, in those crystal structures, all guests were severely disordered, and no atomic positions could be determined.

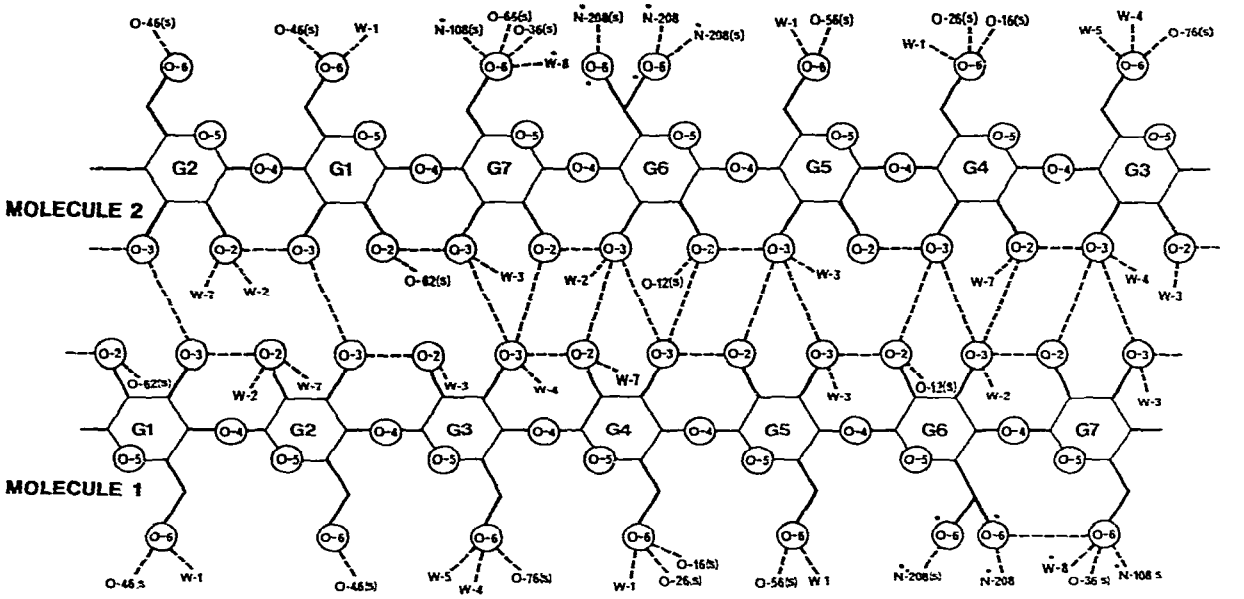
Orientation of the guest. — The benzocaine molecules both lie in the cavity of the cyclomaltoheptaose with their *p*-amino group at the primary-hydroxyl end and their ethyl ester group at the secondary-hydroxyl end. In a previous publication[9], we discussed dimer formation as an analog of "capping" of the cyclomaltoheptaose by chemical modification. Thus, in the crystal, contact of the guest with water through the secondary end is prohibited by the formation of the cyclomaltoheptaose dimer;

TABLE V

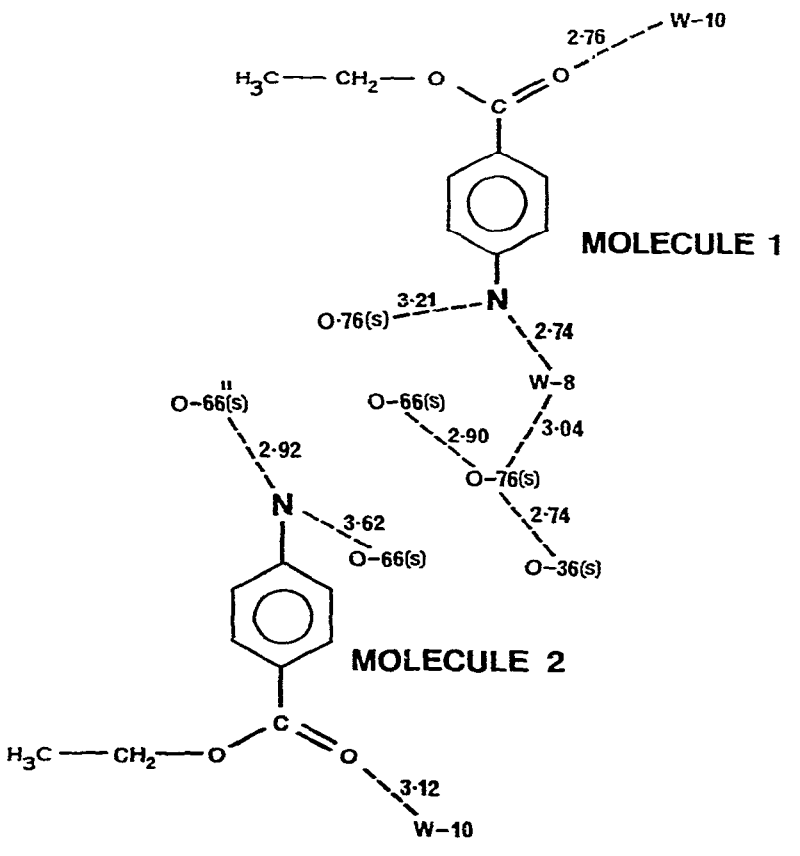
INTERMOLECULAR HYDROGEN-BOND LENGTHS AND ANGLES

Hydrogen bonds involving primary and secondary hydroxyl groups			*Hydrogen bonds involving water molecules*			
Bond	*Distance (pm)*	*Angle (degrees)*	*Bond*	*Distance (pm)*		*Angle (degrees)*
C-12–O-12···O-62	272	102	W-10 V[a]···W-1···O-46 IV[a]	266	286	106
C-13–O-13···O-23	298	113	O-16···W-1···W-10 V	281	266	137
C-22–O-22···W-2	260	117	O-16···W-1···O-46 IV	281	286	102
C-22–O-22···W-7	297	113	O-22···W-2···O-63 V	260	277	128
C-23–O-23···O-13	298	113	O-22···W-2···O-13 III	260	300	65
C-33–O-33···W-4	305	113	O-63 V···W-2···O-13 III	277	300	100
C-33–O-33···O-73	292	114	O-53 V···W-3···W-6	292	278	103
C-42–O-42···W-7	269	107	O-53 V···W-3···O-73 III	292	289	132
C-42–O-42···O-63	297	113	W-6···W-3···O-73 III	278	289	122
C-43–O-43···O-63	276	113	O-33 VI···W-4···O-36 I	305	295	136
C-52–O-52···O-53	297	119	O-33 VI···W-4···W-6 VI	305	278	84
C-53–O-53···W-3	292	115	O-36 I···W-4···W-6 VI	295	278	82
C-62–O-62···O-43	300	113	O-36 I···W-5···W-6 VI	297	263	84
C-62–O-62···O-12	272	115	O-36 I···W-5···O-56 VII	297	277	150
C-63–O-63···W-2	277	118	W-6 VI···W-5···O-56 VII	263	277	117
C-63–O-63···O-43	278	113	W-3···W-6···W-4 VIII	278	278	111
C-72–O-72···O-33	315	117	W-3···W-6···W-5 VIII	278	263	125
C-73–O-73···O-33	292	120	W-4···W-6···W-5 VIII	278	263	93
C-73–O-73···W-3	289	139	O-22···W-7···O-42 VII	279	269	157
C-16–O-16···W-1	281	127	O-76···W-8···N-208	304	274	103
C-26–O-26···O-46	281	103	O-209···W-9···W-10	312	276	97
C-36–O-36···W-4	295	97	O-66′···W-10···W-1 IX	304	266	90
C-36–O-36···W-5	297	102				
C-46–O-46···O-16	289	130				
C-46–O-46···O-26	281	102				
C-46–O-46···W-1	286	97				
C-56–O-56···W-5	277	127				
C-56–O-56···O-56	284	99				
C-66–O-66′···N-208	294	134				
C-66–O-66···N-208	336	110				
C-76–O-76···O-36 I[a]	274	97				
C-76–O-76···O-66	290	116				
C-76–O-76···N-108 I	321	118				
C-76–O-76···W-8	304	100				
C-53–O-53···O-53	290	115				
C-16–O-16···O-46	289	113				

[a]The Roman numerals refer to the following equivalent positions: I, $-x+1, y, -z$; II, $x, y, z+1$; III, $-x+1, y, -z+1$; IV, $-x+1/2, y+1/2, -z$; V, $x-1/2, y+1/2, z$; VI, $x+1, y, z$; VII, $x+1/2, y+1/2, z$; VIII, $x-1, y, z$; and IX, $x+1/2, y-1/2, z$.



a



b

Fig. 2. Hydrogen-bonding scheme of (a) the cyclomaltoheptaose dimer, and (b) the two benzocaine molecules. [G1, G2...G7 represent the residue numbers. The letter (s) represents symmetry-related atoms, and asterisk represents the disordered atoms.]

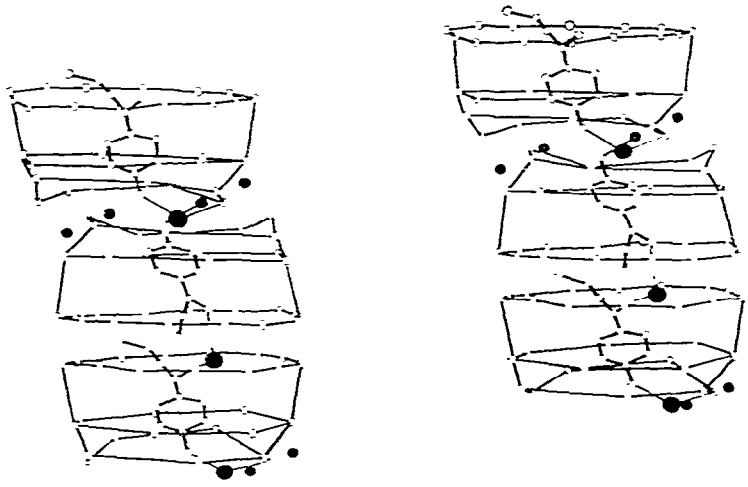


Fig. 3. Stereoview of the dimer plus an additional, complex unit related by cell translation. [The cyclomaltoheptaose molecules are represented by their primary hydroxyl, secondary hydroxyl, and glycosidic oxygen atoms. These are joined to form rings. ● represents the water molecules, and ○, the disordered, primary-hydroxyl atoms.]

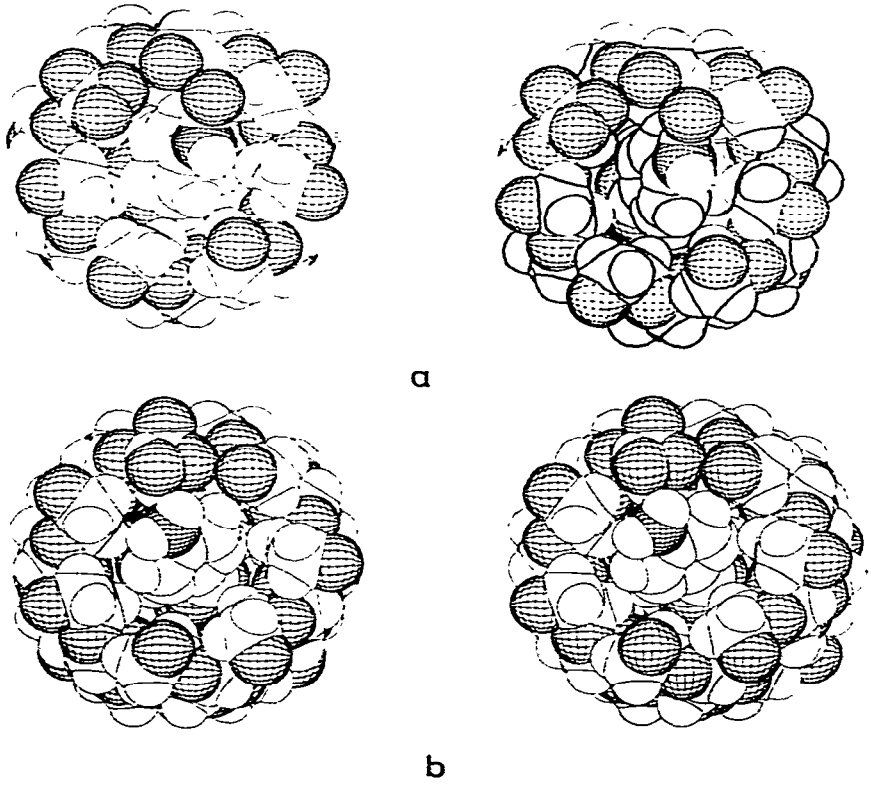


Fig. 4. Stereoview of the space-filling model of the cyclomaltoheptaose complexed with (a) molecule 1, and (b) molecule 2, of the benzocaine guest, seen from the primary-hydroxyl end. [Three of the seven primary-hydroxyl groups point into the cavity, one being disordered.]

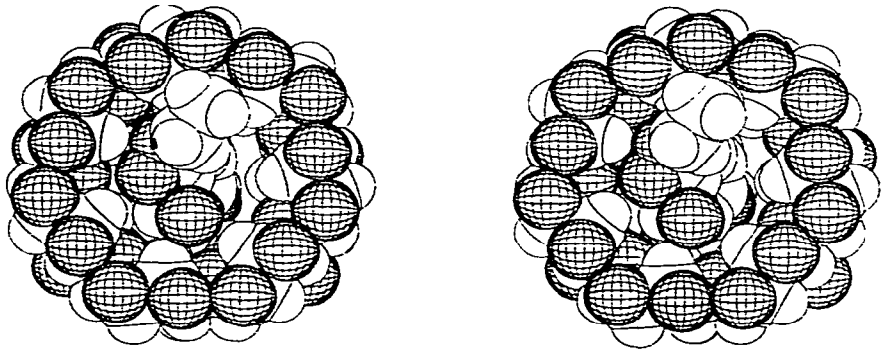


Fig. 5. Stereoview of the complex and the trapped water molecule, viewed from the secondary end.

consequently, the more-polar substituent of the guest, the *p*-amino group, is situated at the primary end of the cyclomaltoheptaose cavity, and is, in fact, hydrogen-bonded, in one case to a primary hydroxyl group, and in the other, to a water molecule (see Figs. 3 and 4). This solvation effect also explained the orientation of other substituted phenol and benzoic acid guests[9] in the crystal form C2. Bergeron *et al.*[11] conducted n.m.r. studies on the binding of a series of carboxylic acids to cyclomaltohexaose, the guests being so chosen that only one-to-one complexes would result, and concluded that solvation of the polar groups is the most important factor in the positioning and stability of the guest in the cavity of the cyclomaltohexaose.

In solution, solvation of the *p*-amino group could be accomplished at either the primary or the secondary end of the cyclomaltoheptaose. The hydrogen-bonding scheme of benzocaine (molecule 2) would be affected by the absence of the dimer, but that of benzocaine (molecule 1), which primarily involves a water molecule in the cavity at the primary-hydroxyl end, could exist in solution. In both cases, the result of the hydrogen bonding of the *p*-amino groups is to pull the benzocaine molecule well down inside the cyclomaltoheptaose cavity, so that the carbonyl oxygen atom of the ethyl ester group is actually below the level of the secondary-hydroxyl oxygen atoms of the cyclomaltoheptaose (see Figs. 3 and 5). There can, therefore, be no interaction with these secondary-hydroxyl groups. Instead, the two carbonyl oxygen atoms of adjacent benzocaine molecules hydrogen-bond to a common water molecule that is trapped inside the cavity near the interface of the secondary hydroxyl groups. The hydrocarbon portions of the ester groups of the benzocaine molecules point to the interior of the cyclomaltoheptaose cavity, are not firmly held, and have high thermal-parameters (see Table I). It is important to realize that, were the crystal structure simply that shown in Fig. 3, the crystal system would be triclinic, with no disorder present. Instead, the converse positioning of the benzocaine molecules in the cyclomaltoheptaose cavity also occurs in the crystal structure in half of the locations, creating statistical disorder. In other words, if the two halves of the cyclomaltoheptaose dimer are repesented by A and B, and the two benzocaine molecules by C and D, dimers of the forms (AC–BD) and (AD–BC) can be present in the crystal, each, overall, in approximately the same proportion.

If it is assumed that the *p*-amino group can hydrogen bond in solution (probably to water, as in the case of benzocaine, molecule 1), so that the overall positioning of, and, more important, penetration into the cyclomaltoheptaose cavity by, the benzocaine is the same in solution as in the crystal, the ester group will lie within the cavity of the cyclomaltoheptaose, and be shielded from attack (see Figs. 3 and 5). There would be no possibility of formation of a tetrahedral intermediate between the benzocaine and the cyclomaltoheptaose as a precursor to possible ester hydrolysis, as suggested by Van Etten *et al.*[4].

The crystal-structure data support the results of the study by Chin *et al.*[5], who found that, whereas hydrolysis of benzocaine is inhibited by cyclomaltohexaose, the hydrolysis of ethyl *o*-aminobenzoate, ethyl *m*-aminobenzoate, and atropine, none of which can be totally included in the cyclomaltohexaose cavity because of steric hindrance, is slightly accelerated by cyclomaltohexaose. Benzocaine can be accommodated in the cyclomaltohexaose cavity. Chin *et al.*[5] concluded that shielding of the carbonyl group and, perhaps, positioning of the carbonyl group away from the secondary hydroxyl groups, was responsible for inhibition of ester hydrolysis by the cyclomaltohexaose. In general, they concluded that the effect of cyclomaltaoses on the alkaline hydrolysis of various esters is one of acceleration or deceleration, depending on the nature of the complexes formed. Compounds that form true inclusion-compiexes, or compounds in which the active center is included in the annulus of the cyclomaltaose, exhibit a deceleration effect, and the rate of hydrolysis is dependent primarily on the amount, in solution, of free ester resulting from dissociation of the complex. Compounds that do not totally fit into the annulus, or are only partially included, leaving the active center sterically fixed in close proximity to the hydroxyl group of the cyclomaltaose, undergo an acceleration effect. In this case, the rate of hydrolysis is due not only to the free ester in solution (resulting from dissociation of the complex), but also to nucleophilic attack of the ester linkage by the alkoxide ion of the cyclomaltaose molecules.

The position of benzocaine in the cyclomaltoheptaose cavity in the crystal certainly supports these conclusions. The whole molecule, including the ester group, lies in the cyclomaltoheptaose annulus, and the carbonyl group of the ester is below the level of the secondary hydroxyl groups of the cyclomaltoheptaose. The agent most responsible for the depth of penetration of the benzocaine, and its stabilization in the complex, is the hydrogen bonding of the *p*-amino groups. Two primary hydroxyl groups (036 and 076) of the cyclomaltoheptaose twist so as to point into the cavity, and thus take part in this hydrogen-bonding scheme. A third primary-hydroxyl group (066) exists in three, alternative positions, one pointing away from the cavity and hydrogen bonding to water in the water channel, and the other two pointing into the cavity and hydrogen bonding to the *p*-amino nitrogen atom of benzocaine molecule 2 (see Figs. 3 and 4). The hydrogen-bonding scheme of each *p*-amino group is separate, and, for the most part, no common atoms are involved. Three primary-hydroxyl groups pointing into the cavity considerably limit the access of solvent at the primary-hydroxyl end. An intramolecular hydrogen-bond (290 pm) is formed

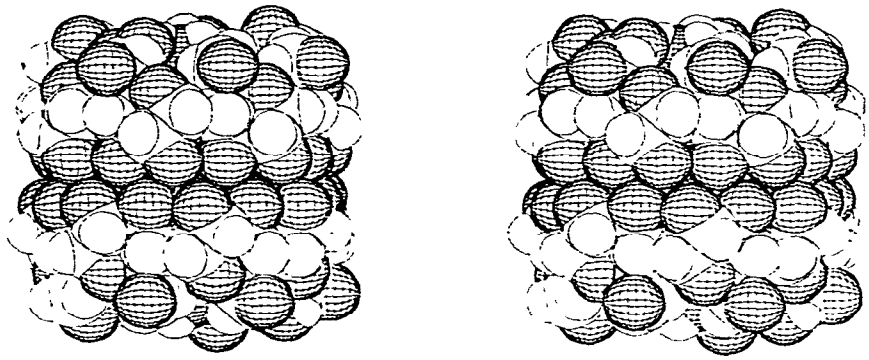


Fig. 6. Stereoview of the dimer, viewed perpendicular to the cavity, showing the tight fit of the cyclomaltoheptaose dimer, and the complete enclosure of the guest.

between two adjacent, primary hydroxyl groups in the *gauche-trans* disposition (076 and 066). This is the first time that such a bond has been observed in the crystal structure of any cyclomaltaose. The cyclomaltoheptaose complex in the crystal is stabilized by an extensive system of intramolecular hydrogen-bonds (see Table IV and Fig. 2a), and also by intermolecular hydrogen-bonds through the water channel (see Table V). The space-filling stereoviews in Figs. 4–6 show the snug fit of the benzocaine in the cyclomaltoheptaose cavity. Fig. 6 also shows the interlocking, secondary-hydroxyl oxygen atoms forming the dimer.

Breslow *et al.*[12] stated that the low increases in rate for cyclomaltaose catalysis *versus* enzyme catalysis is probably due to the substrate's being pulled partially out of the cavity at an intermediate stage in the reaction. In order to improve the rate of catalysis, they used substrates, based on the adamantane framework, that fit more tightly in the cyclomaltoheptaose cavity. An alternative method might involve the use of analogs of benzocaine for which hydrogen bonding of the guest at the primary end of the cyclomaltoheptaose would hold the guest inside the cavity. Benzocaine itself is too short for the essential carbonyl group then to reach the secondary-hydroxyl, oxygen atoms, but the introduction of, perhaps, two carbon atoms between the benzene ring and the carbonyl group should produce a good substrate.

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