Carbohydrate Research, 89 (1981) 33–53
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

STRUCTURE OF CYCLOHEPTAAMYLOSE INCLUSION-COMPLEXES CRYSTAL STRUCTURE OF SUBSTITUTED BENZOIC ACID AND PHENOL DERIVATIVES

JEAN A HAMILTON, MANDAR N SABESAN, AND LARRY K STEINRAUF

Departments of Biochemistry and Biophysics, Indiana University School of Medicine, Indianapolis, Indiana 46223 (U S A)

(Received October 19th 1979, accepted for publication in revised form, June 18th, 1980)

ABSTRACT

Cycloheptaamylose has been crystallized with 2,5-diiodobenzoic acid as guest The X-ray crystal structure at 1 2-Å resolution with space group C2 and cell dimensions a = 19 192 (13), b = 24 759 (20), c = 15 739 (13) Å, and β = 109 6 (3)° was solved by using rotation–translation functions Complexes of other meta-substituted guests were found to be isomorphous, and were solved by using the phases of the cycloamylose of the 2,5-diiodobenzoic acid complex The complex with 2-bromo-5-*tert*-butylphenol having a = 19 235 (11), b = 24 662 (17), c = 16 018 (11) Å and β = 108.9 (2)° was determined at 1 0 Å resolution and the complexes with *m*-bromobenzoic acid, *m*-iodobenzoic acid, *m*-iodophenol, *m*-toluic acid, and 2-bromo-4-*tert*-butylphenol were determined at 2 0-Å resolution In all cases, the guest molecule was disordered However, by using information from all the structures, it may be concluded that the functionally important carboxylic acid group lies in the primary-hydroxyl end of the cycloheptaamylose molecule As studies in solution have shown that the hydrogen-bonding groups of guest molecules interact with the secondary-hydroxyl end of the cycloheptaamylose molecule, it is concluded that the structure seen in the crystals here does not correspond to a catalytically active species Cycloheptaamylose exists as a dimer in the crystal by means of extensive hydrogen bonding across the secondary-hydroxyl ends of two cycloheptaamylose molecules A continuous channel throughout the crystal is achieved by the stacking of these dimer units

INTRODUCTION

The cycloamyloses (Schardinger dextrins) are cyclic oligosaccharides containing 6–12 D-glucopyranosyl residues linked α-(1→4), the most common have 6, 7, or 8 residues They are produced[1] from starch by exoenzymes of *Bacillus macerans* or *Bacillus megaterium* The cycloamyloses form complexes with iodine, as does starch, which gives the well-known blue coloration From knowledge of the structure of the cyclohexaamylose–iodine complex, a structure has been proposed for that of the starch-iodine complex[2]

0008-6215/81/0000–0000/$ 02 50,

The cycloamyloses are able to form inclusion complexes with a wide variety of "guest" molecules, provided that the guest is small enough to fit into the central cavity of the cycloamylose[3] In addition the cycloamyloses have been shown to cause a remarkably stereoselective acceleration of the cleavage of phenyl esters and phosphoric esters in homogeneous aqueous solutions[3], and it was found that cyclohexa- and cyclohepta-amylose (previously also called α-dextrin and β-dextrin) were the best catalysts, but often having different specificities[3,4]. The mechanism of hydrolysis of phenyl esters was shown to involve the complexed ester molecule and the alkoxide ion derived from the secondary hydroxyl groups of the cycloamylose[4] These observations have led to the cycloamyloses being used as models for enzyme catalysis

Recent research has shown that structural modifications of the cycloamyloses can improve the catalytic abilities[5] and has expanded the scope of the reactions catalyzed by the cycloamyloses[7] In addition, these studies[5,7] have made it clear that the effectiveness with which the cycloamyloses catalyze a reaction is related to the position of the guest in the cavity of the cycloamylose and to the strength of the binding of the guest In an effort to improve the binding and catalytic efficiency of the cycloamyloses, "capped" derivatives of cycloamyloses have been synthesized[5,6] These caps are hydrophobic molecules, covalently bound to one or more of the hydroxyl groups at one end of the cycloamylose molecule The function of the cap is to restrict access of water to one end of the cycloamylose molecule and thereby to improve the stability of the hydrophobic guest It was the observations of Bender[8] and Breslow[5] on these geometric factors and the knowledge that the best catalytic rates have been shown to be obtained with cycloheptaamylose and *m*-substituted phenolic esters[8] that prompted us to undertake a study of cycloheptaamylose complexes with *m*-substituted aromatic guests

The first X-ray crystal structure of a cycloamylose, that of cyclohexaamylose with the disordered guest potassium acetate, was determined by Hybl, Rundle, and Williams[9] in 1965 Since then, the X-ray crystal structures of several other complexes with cyclohexaamylose have been determined by Saenger and co-workers[10] and by Harata[11] The first X-ray structure of cycloheptaamylose, that with the guest 2,5-diiodobenzoic acid, was reported in a preliminary announcement by us[12] The present work describes results on an isomorphous series of seven different guests with cycloheptaamylose 2,5-diiodobenzoic acid, *m*-toluic acid, *m*-bromobenzoic acid, *m*-iodobenzoic acid, *m*-iodophenol, 2-bromo-4-*tert*-butylphenol, and 2-bromo-5-*tert*-butylphenol While these guests are not substrates for cycloheptaamylose, they can be products (*m*-chlorophenyl benzoate has been used as a substrate) and all are inhibitors of catalysis by cycloheptaamyloses

EXPERIMENTAL

The crystal data for the complexes with 2,5-diiodobenzoic acid and with 2-bromo-4-*tert*-butylphenol are given in Table I All crystals were prepared by the method given previously[13], data for all other crystal forms are to be found therein

TABLE I

CRYSTAL DATA OF CYCLOHEPTAAMYLOSE COMPLEXES

	2,5-Diiodo-benzoic acid complex	*2-Bromo-4-tert-butylphenol complex*
Crystal system	monoclinic	monoclinic
Space group	C2	C2
Z	4	4
Observed density (g/cm^3)	1 60	1 41
Cell dimensions (Å) a	19 192(13)	19 235(11)
b	24 759(20)	24 662(17)
c	15 739(13)	16 018(11)
β	109 6(3)°	108 9(2)°
Cell volume (Å^3)	7045 4	7188 8

Crystals became opaque through loss of water and therefore had to be sealed in quartz capillary tubes Complete three-dimensional data, consisting of 4 400 unique reflections, were collected for the 2,5-diiodobenzoic acid complex with a Nonius four-circle diffractometer with CuKα radiation at the University of Leeds (England) Of these, the 1,883 observed reflections extending to 1 2-Å resolution were used in the structure determination and refinement, reflections beyond that resolution were too weak to be observed

Diffraction data from the 2-bromo-4-*tert*-butylphenol complex consisting of 3,287 observed unique reflections extending to 1 0-Å resolution were collected on a Syntex $P2_1$ diffractometer with CuKα radiation Data to 2 0-Å resolution for the other complexes were collected with a Supper–Pace diffractometer with MoKα radiation In all cases, care was taken to choose a small nearly spherical crystal to minimize errors due to absorption no corrections for absorption were therefore applied

The 2 0-Å data were collected first and were intended to be used for a structure determination by isomorphous replacement However, a study of the data revealed that the series was not suitable for application of the isomorphous-replacement method because of extensive disordering of the guest molecules The data in conjunction with the solved complex of 2,5-diiodobenzoic acid, later allowed us to draw conclusions as to the position of the guest molecules in the cavity

STRUCTURE DETERMINATION

Chemical analyses of the cycloheptaamylose complexes were consistent with 1 1 complexes with the guest molecules[13] However, the Patterson and difference-Patterson maps were totally uninterpretable in terms of any reasonable occupancy of a limited number of heavy-atom sites We therefore concluded that the guest molecules, and consequently the heavy atoms themselves, were highly disordered

making the heavy-atom methods and the isomorphous-replacement methods not applicable At this point, our strategy was shifted to the rotation–translation search methods[14 15]

The orientation of the cycloheptaamylose molecule in the unit cell requires the determination of three translational variables and three rotational variables

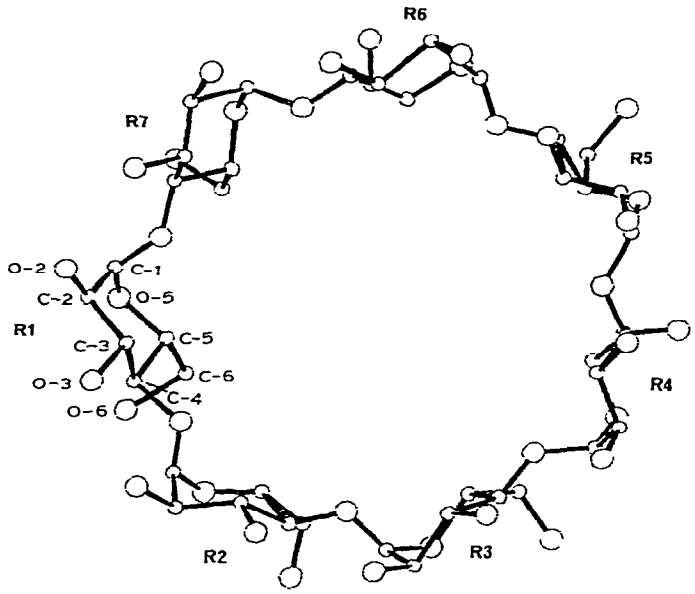


Fig 1 Numbering scheme of the cycloheptaamylose molecule R1, R2, R7 denote the residue numbers

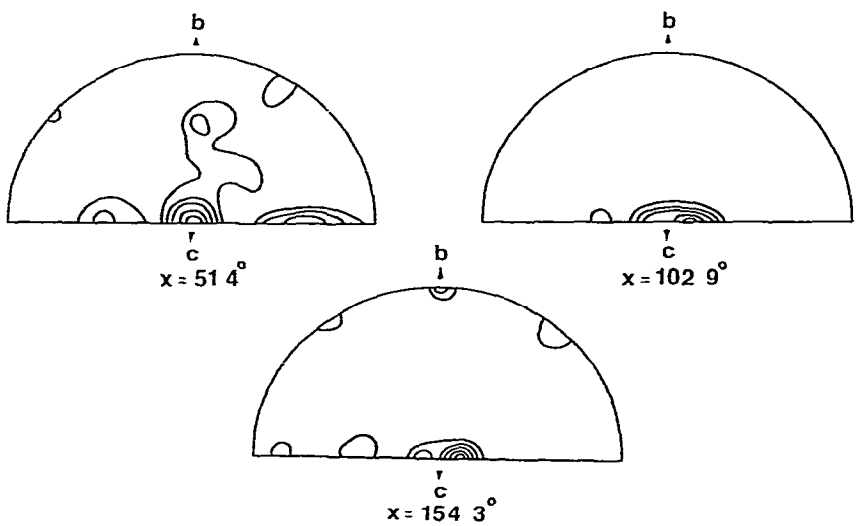


Fig 2 Stereographic projection of the rotation function Only reflections to 4 0-Å resolution were used in this calculation

($X,Y,Z,\phi,\psi,0$) The molecule has seven D-glucosyl residues in a cyclic arrangement (Fig 1), and from the crystal-structure studies[9,10] of cyclohexaamylose it was reasonable to assume that the complexed molecule would have approximately sevenfold symmetry The direction of this non-crystallographic, seven-fold axis was determined by using the rotation function of Rossmann and Blow[15] The asymmetric unit of the rotation function in spherical polar coordinates is ϕ = 0–180°, ψ = 0–180°, and χ = 0–360° As the search was for a nonc-rystallographic, seven-fold axis, it was necessary to calculate the function only for χ = 1/7, 2/7, and 3/7 of 360°, χ = 4/7, 5/7, and 6/7 of 360° are related by symmetry[16] Fig 2 shows the three stereographic projections of the rotation function calculated with reflections to 4 0-Å resolution The strongest peak in the three sections of χ consistently occurred at ϕ = 90 ±2° and ψ = 100 ±2°, thereby giving the direction of the seven-fold axis However, as the rotation function, like the Patterson function, is centrosymmetric, it cannot distinguish between the direction of the primary-hydroxyl end and the secondary-hydroxyl end of the cycloheptaamylose molecule This ambiguity is resolved later when we introduce the additional rotational variable, σ A closer search with higher-resolution data did not shift the position of the peak The direction of the seven-fold axis turned out to be only 10° away from the crystallographic c axis

In fact the precession photographs down the c axis did give some indication of seven-fold rotational symmetry among the low-order reflections, however a translation search on the basis of that approximate orientation had not given meaningful results Thus a precise determination of the molecule, particularly in the case of "small molecules ', is essential to the structure determination by rotation–translation methods

Having accurately determined the orientation of the molecular, seven-fold axis, the next step was the determination of the translation parameters[14] Only the two translation parameters along the x and z directions are necessary the origin is arbitrary along y In order to determine the translation parameters by search methods, it is necessary to have a trial model for the molecule This was obtained by computer modeling, starting with the fractional coordinates of one D-glucosyl residue[9] and using a seven-fold rotation matrix to generate a seven-residue cycloamylose molecule with reasonable bond distances and angles The primary-hydroxyl oxygen atoms were excluded from the model, because of the possible freedom of rotation around C-5–C-6 (see Fig 1) This model was given that orientation with respect to the unit-cell axes, as determined by the rotation-function calculations In order to determine the position of this properly oriented molecule in the unit cell it is necessary to vary four search parameters These are (*1* and *2*) the translational parameters along x and z, (*3*) the angle of rotation of the cycloheptaamylose model (angle θ in Fig 3) around the known direction of the seven-fold axis, and (*4*) the choice between the two possible arrangements related by 180° rotation about an axis perpendicular to the seven-fold axis (α = 0 or 180°) The conventional, R-index was calculated for all possible values of these parameters X = 0 to $a/2$, Z = 0 to $c/2$, θ = 0 to 360/7°, and α = 0 or 180° Only data to 3 0-Å resolution were used in the search The results gave an R-index

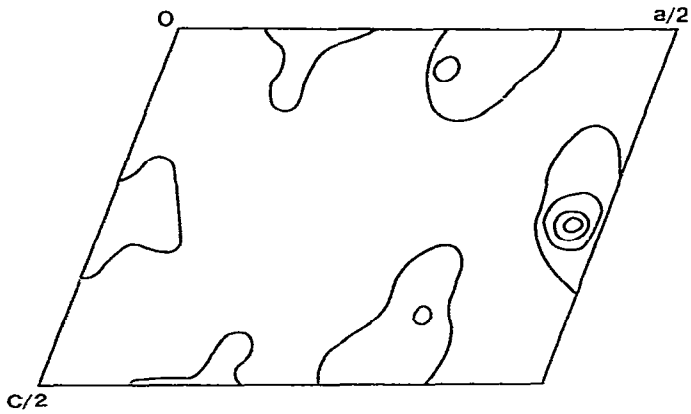


Fig 3 The section at $\theta = 37.8°$ and $\varphi = 0°$ showing the highest peak in the R-factor search which gives the position of the cyclohepta-amylose molecule in the unit cell

minimum of 0.58 at $X = 0.470$, $Z = 0.260$ $\theta = 37.8°$, and $\sigma = 0°$ (Fig 3) The next minimum was 0.70 and the background average was 0.75

Refinement — The coordinates of all atoms contributing to the R-index minimum in the translation search just described were then subjected to block-diagonal, least-squares refinement with all observed data The difference-Fourier map showed the primary hydroxyl oxygen atoms and a number of water molecules of both full and partial occupancy The subsequent difference-Fourier map showed the iodine sites of the 2,5-diiodobenzoic acid guest to be disordered around the seven-fold axis (see Fig 5) Difference-Fourier maps calculated from the 2.0-Å data of the *m*-iodobenzoic acid the *m*-bromobenzoic acid, and the *m*-toluic acid complexes of cycloheptaamylose showed the primary hydroxyl and water oxygen atoms coincident with those obtained from the data for the diiodobenzoic acid complex Several cycles of least-squares refinement, combined with difference-Fourier maps using the complete cycloheptaamylose molecule 10 partly occupied iodine sites and 8 water molecules in the phasing calculations, brought the R-index to 0.19 for the 1 885 reflections observed for the diiodobenzoic acid complex

The quantity minimized in the least-squares refinement was

$$\sum(|F_o| - |F_c|)^2$$

The atomic scattering-factors and anomalous-dispersion corrections for the iodine atoms were taken from the International Tables for X-Ray Crystallography[17] The X-ray 72 system of computer programs was used for least-squares refinement and Fourier synthesis

The refined coordinates of the cycloheptaamylose molecule (excluding the primary hydroxyl oxygen atoms) from the foregoing determination were used to provide a starting model for the refinement of the data for the complex with 2-bromo-4-*tert*-butylphenol Several cycles of least-squares refinement, combined with difference-Fourier maps, were used to locate the primary hydroxyl and water oxygen

TABLE II

FRACTIONAL COORDINATES AND THERMAL PARAMETERS[a] OF NON-HYDROGEN ATOMS[b] ALL VALUES ARE MULTIPLIED BY 10^4

Atom	x	y	z	*U*	*Atom*	x	y	z	*U*
a 2-Bromo-4-*tert*-butylphenol–cycloheptaamylose complex									
C-11	7183(14)	1729(10)	3147(15)	3 4	C-41	1444(16)	−786(11)	1887(18)	4 9
C-12	7015(14)	2012(11)	3875(17)	4 2	C-42	1679(16)	−1144(11)	2730(19)	5 4
C-13	6206(15)	1958(11)	3796(17)	4 5	C-43	2426(14)	−1324(10)	2992(15)	3 2
C-14	5760(14)	2157(10)	2899(15)	3 3	C-44	2537(17)	−1636(12)	2241(18)	6 0
C-15	5973(14)	1887(10)	2144(16)	3 8	C-45	2332(16)	1255(11)	1395(18)	4 8
C-16	5636(18)	2136(13)	1264(21)	6 4	C-46	2319(16)	−1548(11)	509(18)	5 1
O-12	7460(11)	1856(8)	4723(12)	5 4	O-42	1514(11)	−845(8)	3437(13)	6 1
O-13	6041(11)	2270(8)	4455(12)	5 3	O-43	2579(10)	−1699(7)	3705(11)	5 1
O-14	4986(10)	2015(7)	2742(11)	4 5	O-44	3290(10)	−1745(7)	2424(11)	4 5
O-15	6748(9)	1921(7)	2331(11)	4 2	O-45	1565(10)	−1056(7)	1207(11)	4 8
O-16	5735(12)	2715(8)	1246(13)	6 7	O-46	1753(12)	−1999(8)	344(13)	6 4
C-21	4340(17)	2473(12)	2408(19)	5 6	C-51	3466(17)	−2294(12)	2313(19)	5 8
C-22	4017(15)	2526(11)	3100(16)	4 3	C-52	3946(16)	−2489(12)	3141(18)	5 5
C-23	3599(14)	1991(10)	3130(16)	3 8	C-53	4718(14)	−2200(10)	3442(16)	3 5
C-24	3126(15)	1900(11)	2268(17)	4 5	C-54	5075(16)	−2219(12)	2700(18)	5 2
C-25	3563(15)	1833(10)	1606(17)	4 1	C-55	4503(20)	−1968(15)	1850(22)	7 7
C-26	3054(23)	1754(17)	625(26)	9 8	C-56	4775(24)	−1957(18)	1031(27)	10 3
O-22	4545(11)	2608(8)	4009(12)	5 8	O-52	3653(11)	−2487(8)	3826(12)	6 1
O-23	3178(10)	2065(7)	3767(12)	5 1	O-53	5201(11)	−2416(9)	4217(13)	6 7
O-24	2722(9)	1380(7)	2270(10)	3 6	O-54	5681(11)	−1886(8)	2959(12)	5 6
O-25	3946(10)	2339(7)	1644(11)	5 1	O-55	3827(11)	−2308(8)	1632(13)	6 6
O-26	2599(17)	2185(12)	313(18)	11 6	O-56	4948(21)	−2499(17)	1027(25)	16 7
C-31	1947(17)	1380(12)	1897(19)	5 2	C-61	6395(16)	−2109(11)	2916(17)	4 6
C-32	1647(17)	1154(12)	2564(19)	5 8	C-62	6933(16)	−2038(11)	3806(18)	5 1
C-33	1942(16)	538(11)	2817(18)	4 8	C-63	7091(16)	−1420(12)	3913(18)	5 1
C-34	1643(15)	232(10)	1918(16)	3 9	C-64	7331(13)	−1216(9)	3220(15)	3 2
C-35	1977(16)	500(11)	1328(18)	5 1	C-65	6732(17)	−1328(12)	2325(19)	5 6
C-36	1697(22)	228(16)	382(24)	8 6	C-66	6901(23)	−1224(17)	1429(26)	17 5
O-32	1885(12)	1474(8)	3377(13)	6 3	O-62	6717(10)	−2256(7)	4526(11)	5 0
O-33	1573(11)	313(8)	3429(12)	5 5	O-63	7679(11)	−1332(8)	4759(13)	6 1
O-34	1976(9)	−294(7)	2167(10)	4 0	O-64	7330(11)	−623(8)	3294(13)	5 9
O-35	1715(10)	1037(7)	1124(11)	4 7	O-65	6600(11)	−1918(8)	2247(12)	5 5
O-36	945(16)	194(11)	−15(17)	10 1	O-66	7506(37)	−1474(29)	1497(42)	20 9
C-71	8000(17)	−359(12)	3342(19)	5 7	O-W1[c]	5000(0)	4362(18)	0000(0)	12 1
C-72	8241(17)	−17(12)	4095(18)	5 7	O-W2	3967(19)	3916(18)	2026(21)	13 5
C-73	7747(15)	456(11)	4084(17)	4 5	O-W3	4531(12)	3445(9)	801(13)	6 9
C-74	7614(15)	790(11)	3274(17)	4 2	O-W4	4071(19)	3480(14)	4824(22)	14 2
C-75	7274(19)	422(14)	2427(21)	7 1	O-W5	1431(26)	2538(21)	689(31)	21 8
O-76	7231(33)	742(25)	1585(38)	15 6	O-W6	748(29)	1526(23)	3857(33)	23 6
O-72	8391(12)	−323(9)	4881(13)	6 6	O-W7	9143(21)	49(16)	1749(23)	16 1
O-73	8045(12)	802(9)	4853(14)	7 1	O-W8	8483(40)	1551(32)	689(46)	32 4
O-74	7032(10)	1170(7)	3215(11)	4 5	O-W9	9098(33)	1480(25)	4590(37)	26 2
O-75	7891(13)	−32(9)	2566(14)	7 4	Br-1[d]	5000(0)	794(10)	0000(0)	20 3
O-76	8123(21)	951(15)	1755(23)	15 2	Br-2	5000(0)	1190(15)	5000(0)	9 7
					Br-3	3677(27)	782(22)	4828(31)	25 3
					Br-4	3415(24)	−257(18)	4725(27)	12 5

TABLE II *(continued)*

Atom	*x*	*y*	*z*	*U*	*Atom*	*x*	*y*	*z*	*U*
b 2,5-Diiodobenzoic acid–cycloheptaamylose complex									
C-11	7066(29)	1673(21)	3121(34)	4 2	C-41	1476(42)	−0746(32)	1963(53)	9 2
C-12	6959(24)	2041(18)	3908(27)	1 0	C-42	1677(32)	−1151(24)	2751(39)	5 0
C-13	6186(27)	1969(21)	3764(32)	2 9	C-43	2368(32)	−1298(26)	3043(40)	6 1
C-14	5606(27)	2162(21)	2846(32)	2 7	C-44	2520(25)	−1651(20)	2244(30)	1 3
C-15	5979(30)	1870(23)	2146(37)	4 3	C-45	2149(26)	−1198(20)	1348(32)	2 5
C-16	5569(27)	2156(21)	1150(32)	3 0	C-46	2205(37)	−1555(31)	498(48)	8 7
O-12	7421(18)	1835(14)	4719(21)	3 3	O-42	1542(24)	−877(19)	3482(29)	7 5
O-13	5950(19)	2276(15)	4476(22)	4 1	O-43	2544(18)	−1695(15)	3662(22)	4 3
O-14	4907(15)	2023(12)	2695(18)	1 5	O-44	3244(16)	−1769(13)	2435(20)	2 9
O-15	6669(19)	1911(14)	2240(23)	4 3	O-45	1491(19)	−1071(14)	1156(23)	4 0
O-16	5764(25)	2699(19)	1220(30)	8 6	O-46	1715(21)	−1994(16)	358(26)	5 9
C-21	4436(28)	2397(21)	2380(34)	2 1	C-51	3455(30)	−2246(23)	2349(36)	3 8
C-22	4010(35)	2543(28)	3083(42)	6 1	C-52	4023(28)	−2601(22)	3325(34)	3 4
C-23	3628(24)	2038(18)	3146(28)	1 1	C-53	4706(29)	−2178(22)	3450(34)	3 0
C-24	3056(32)	1879(25)	2224(39)	5 6	C-54	5038(33)	−2244(25)	2688(40)	4 6
C-25	3431(29)	1851(22)	1499(34)	4 2	C-55	4366(32)	−2060(24)	1770(38)	4 5
C-26	2954(49)	1788(37)	570(59)	11 3	C-56	4752(37)	−2076(27)	926(44)	7 6
C-22	4509(21)	2605(17)	3986(26)	5 9	O-52	3637(19)	−2474(15)	3847(23)	4 1
O-23	3141(19)	2060(15)	3786(23)	4 4	O-53	5204(15)	−2376(12)	4207(19)	1 6
O-24	2682(19)	1410(15)	2255(23)	4 0	O-54	5596(17)	−1929(12)	2815(20)	2 2
O-25	3881(19)	2369(15)	1592(23)	4 5	O-55	3734(19)	−2353(15)	1602(24)	4 8
O-26	2469(29)	2287(22)	338(33)	10 5	O-56	4879(34)	−2607(26)	979(40)	16 3
C-31	1924(26)	1351(20)	1867(31)	1 9	C-61	6293(26)	−2118(20)	2778(31)	1 4
C-32	1604(28)	1155(21)	2562(34)	2 8	C-62	6951(27)	−1996(20)	3759(33)	2 1
C-33	1899(31)	595(24)	2726(36)	4 4	C-63	7024(26)	−1415(21)	3871(32)	2 4
C-34	1574(28)	254(22)	1904(33)	3 9	C-64	7207(30)	−1170(22)	3093(37)	4 1
C-35	1981(41)	527(32)	1292(49)	9 7	C-65	6510(28)	−1306(22)	2208(35)	3 6
C-36	1648(32)	235(25)	0355(38)	4 9	C-66	6651(41)	−1105(32)	1147(50)	9 0
O-32	1777(19)	1475(15)	3309(23)	4 6	O-62	6687(19)	−2254(14)	4408(22)	4 2
O-33	1525(17)	329(13)	3412(20)	3 0	O-63	7586(21)	−1289(17)	4645(26)	6 2
O-34	1883(22)	−234(18)	2165(27)	7 0	O-64	7275(22)	−624(16)	3185(28)	5 4
O-35	1695(19)	1032(15)	1055(23)	3 9	O-65	6474(21)	−1907(16)	2163(26)	5 3
O-36	1004(24)	191(19)	−0022(28)	7 5	O-66	7227(50)	−1404(39)	133(62)	22 8
C-71	7842(33)	−341(26)	3195(40)	5 9	O-W1	5200(38)	4442(24)	84(60)	4 6
C-72	8165(30)	−37(25)	3978(36)	4 8	O-W2	4003(32)	3985(25)	2054(39)	13 2
C-73	7667(29)	459(22)	4121(34)	3 2	O-W3	4532(23)	3449(17)	755(27)	7 5
C-74	7538(28)	802(22)	3092(34)	3 3	O-W4	4069(36)	3426(28)	4698(44)	17 2
C-75	7187(33)	394(25)	2341(38)	5 6	O-W5	9873(51)	729(40)	492(60)	10 6
C-76	6976(50)	689(40)	1226(60)	13 8	O-W6	4919(46)	4060(36)	3505(56)	20 7
O-72	8366(21)	−294(17)	4775(26)	5 6	O-W7	9190(35)	51(30)	1791(41)	7 7
O-73	8002(20)	790(16)	4808(24)	5 6					
O-74	6961(19)	1163(15)	3190(24)	5 1					
O-75	7749(27)	0000(23)	2463(31)	9 5					
O-76	7756(39)	1074(31)	1535(49)	17 6					

TABLE II *(continued)*

Atom No	*x*	*y*	*z*	*PP*	*U11*	*U22*	*U33*	*U12*	*U23*	*U13*
I-1[e]	4609(9)	676(8)	994(9)	0 48	2011	1469	1161	−168	517	−86
I-2	3728(10)	−249(8)	4612(12)	0 39	1387	1330	1399	−244	424	−304
I-3	4687(30)	−610(20)	964(26)	0 21	3421	1956	1340	1648	693	344
I-4	3698(24)	270(19)	4420(22)	0 10	1340	707	167	428	248	211
I-5	4172(26)	759(17)	4987(29)	0 16	1868	974	1544	−94	547	−509
I-6	4106(23)	−671(17)	4738(26)	0 25	2504	1639	2471	−501	945	125
I-7	5123(24)	362(17)	1007(23)	0 13	1195	1011	0905	−169	182	−91
I-8	3895(20)	−413(16)	1026(23)	0 28	2100	2127	2102	220	1311	510
I-9	4740(36)	989(21)	4985(58)	0 10	1309	0758	1889	−26	1560	−167
I-10	3702(45)	206(33)	769(46)	0 08	1742	1805	0952	1484	1175	1016

[a]The anisotropic temperature-parameters are of the form $\exp[2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2hka^*b^*U_{12} + 2hla^*c^*U_{13} + 2klb^*c^*U_{23})]$ [b]The numbering 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) [c]W = water molecule [d]The population parameters of Br-1 Br-2 Br-3 and Br-4 are 0 55 0 16 0 23 and 0 13 respectively [e]I = disordered iodine atoms, PP = population parameter

atoms The guest molecule, however, still showed disorder but of a type different from that seen before Also, the water molecule (W-1) situated on a crystallographic, two-fold axis was more clearly seen to be disordered

The final R-index with 77 non-hydrogen atoms of the cycloheptaamylose molecule, 9 water molecules, and 4 disordered bromine atoms, was 0 18 with 3 287 reflections The final atomic coordinates and temperature factors for both the 2 5-diiodobenzoic acid complex and the 2-bromo-4-*tert*-butylphenol complex are given in Table II

RESULTS AND DISCUSSION

Crystal structure — The cycloheptaamylose molecules are stacked together like coins in a roll, with alternating heads and tails up (Fig 4) The crystallographic 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 cycloheptaamylose molecules are bonded directly together by hydrogen bonds The primary ends are farther apart and are connected by hydrogen-bonded water molecules The cycloheptaamylose columns are connected side-by-side by water molecules, which form narrower columns parallel to the cycloheptaamylose columns Within the cycloheptaamylose column is a continuous channel wherein the disordered guests are found The tight contact between the secondary ends gives the appearance of a dimer of cycloheptaamylose This dimerization has the effect of excluding all water from the secondary-hydroxyl end, and is in fact quite contrary to what would be expected in solution

The crystallographic refinement was severely limited by the disorder of the

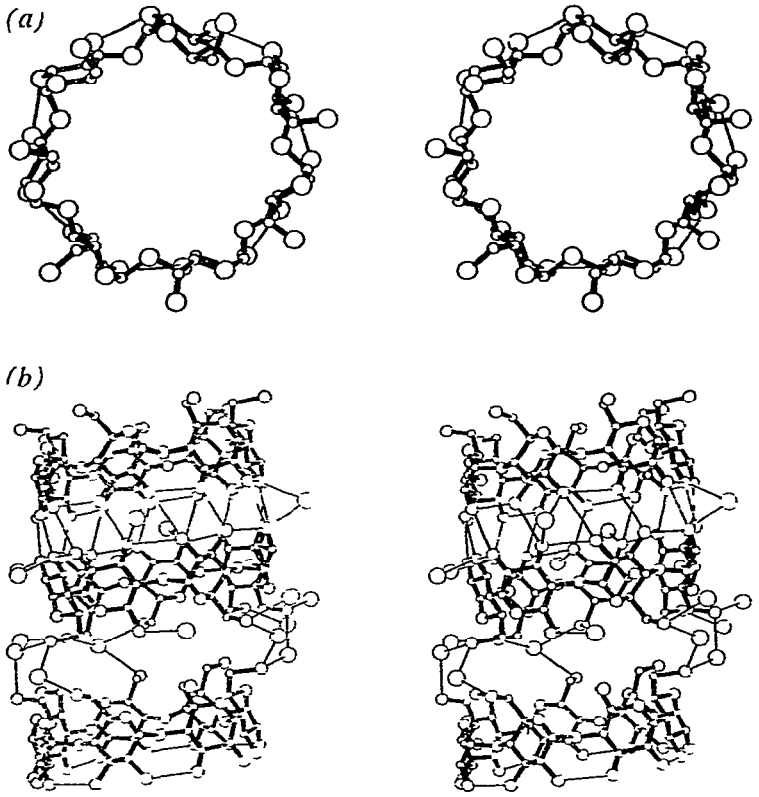


Fig 4 (*a*) Stereo view of the cycloheptaamylose molecule looking down the cavity (*b*) Stereo view of channel formation by the cycloheptaamylose molecules in the crystal

guest molecules It was not possible to obtain a good fit even for the heavy atoms Because of this, the accuracy of the determination of the atoms of the cycloheptaamylose and the water molecules is lower than usual for a structure of this size Data for the 2-bromo-4-*tert*-butylphenol complex are better than those for the 2,5-diiodobenzoic acid complex, and so bond lengths, bond angles, and dihedral angles are given for the former only Bond distances and angles are given in Table III These are in general agreement with the values observed for the cyclohexaamylose structures[10,18]. The seven-fold symmetry of the molecule is maintained within the experimental limits of accuracy, which explains why our use of the rotation function was so successful The dihedral angles are given in Table IV The seven D-glucosyl residues are in the convention $^4C_1$ chair conformation All of the primary hydroxyl groups point away from the center of the molecule and are related by the seven-fold axis (Fig 4) Tables V and VI give intra- and inter-molecular hydrogen-bond data

Orientation of the guests — The difference maps using the independent datasets from each of the seven isomorphous complexes of cycloheptaamylose, with the contribution of cycloheptaamylose and the water molecules subtracted, show exten-

TABLE III

BOND LENGTHS (Å) AND ANLES DEGREES OF THE CYCLOHEPTAAMYLOSE MOLECULE

Bond	*G1*	*G2*	*G3*	*G4*	*G5*	*G6*	*G7*
Lengths							
C-1–C-2	1 48(4)	1 44(5)	1 48(5)	1 56(3)	1 43(4)	1 48(3)	1 42(4)
C-1–O-5	1 39(3)	1 26(3)	1 44(3)	1 36(4)	1 47(4)	1 34(4)	1 44(4)
C-2–C-3	1 53(4)	1 55(4)	1 63(4)	1 43(4)	1 58(4)	1 55(4)	1 50(4)
C-2–O-2	1 40(3)	1 50(3)	1 46(4)	1 46(4)	1 39(4)	1 45(4)	1 42(4)
C-3–C-4	1 50(3)	1 40(3)	1 56(4)	1 50(4)	1 55(5)	1 43(4)	1 49(4)
C-3–O-3	1 42(4)	1 50(4)	1 49(4)	1 42(3)	1 39(3)	1 47(3)	1 45(3)
C-4–C-5	1 55(4)	1 56(5)	1 46(4)	1 59(4)	1 57(4)	1 55(3)	1 59(4)
C-4–O-4	1 47(3)	1 50(3)	1 44(3)	1 41(4)	1 38(4)	1 47(3)	1 44(3)
C-5–O-5	1 42(3)	1 44(3)	1 42(3)	1 49(4)	1 49(4)	1 48(4)	1 59(4)
C-5–C-6	1 48(4)	1 57(4)	1 58(5)	1 59(4)	1 56(6)	1 59(6)	1 54(7)
C-6–O-6	1 44(4)	1 37(5)	1 38(5)	1 52(4)	1 38(6)	1 29(9)	1 73(7)
O-4–C -1[a]	1 64(3)	1 42(3)	1 56(3)	1 42(4)	1 50(4)	1 42(4)	1 42(3)
Angles							
C-1–C-2–C-3	112(2)	108(2)	110(3)	114(3)	113(3)	106(2)	115(2)
C-1–C-2–O-2	114(2)	116(2)	111(2)	108(2)	115(3)	116(2)	111(3)
C-2–C-3–C-4	108(2)	106(2)	103(2)	109(2)	111(2)	111(3)	112(3)
C-2–C-3–O-3	111(2)	108(2)	108(2)	111(2)	113(2)	108(2)	112(2)
O-2–C-2–C-3	110(2)	106(2)	107(2)	113(2)	110(2)	112(3)	112(3)
C-3–C-4–C-5	113(2)	111(2)	106(2)	109(2)	107(3)	109(2)	110(2)
C-3–C-4–O-4	109(2)	108(2)	101(2)	110(2)	107(2)	106(2)	109(2)
O-3–C-3–C-4	110(2)	112(2)	108(2)	105(2)	110(2)	109(2)	109(2)
O-4–C-4–C-5	106(2)	108(2)	110(2)	104(3)	108(2)	103(2)	103(2)
C-4–C-5–O-5	110(2)	105(2)	112(3)	109(2)	107(3)	108(2)	102(2)
C-4–C-5–C-6	115(2)	113(3)	110(3)	115(2)	114(3)	120(3)	110(3)
C-5–C-6–O-6	114(2)	113(3)	117(3)	107(2)	98(4)	107(4)	104(4)
C-5–O-5–C-1	117(2)	114(2)	111(2)	114(2)	113(2)	111(2)	114(3)
O-5–C-5–C-6	106(2)	107(2)	101(2)	104(2)	108(3)	99(3)	108(4)
O-5–C-1–C-2	111(2)	120(3)	108(2)	110(2)	111(3)	116(2)	109(2)
C-4–O-4–C′-1*	121(2)	118(2)	115(2)	115(2)	118(2)	116(2)	118(2)
O-4–C′-1–C′-2	106(2)	107(2)	102(2)	108(2)	106(2)	111(3)	107(2)
O-4–C′-1–O′-5	107(2)	111(2)	110(2)	108(2)	115(2)	109(2)	109(2)

[a]The primed numbers refer to atoms on neighboring residues

sive electron density only within the cavity, usually at locations quite reasonable for guest molecules disordered into seven positions related by rotation about the seven-fold axis but possibly continuously distributed through a volume of space Based on the assumption that the heavy atoms of the guests would dominate in the electron density of the disordered region, it was possible to establish the general features of the guests Although we could not determine the possible orientations of the guest molecule in the cavity by looking at a single difference-Fourier map, we could make definite conclusions about the location of the catalytically important carboxyl group when we compared difference Fourier-maps using all of the in-

TABLE IV

DIHEDRAL ANGLES OF THE CYCLOHEPTAAMYLOSE MOLECULE

Dihedral angle (deg)	*G1*	*G2*	*G3*	*G4*	*G5*	*G6*	*G7*
C-1–C-2–C-3–C-4	−55	−53	−60	−58	−52	−56	−55
C-2–C-3–C-4–C-5	52	61	61	58	55	59	57
C-3–C-4–C-5–O-5	−51	−61	−67	−56	−60	−58	−58
C-4–C-5–O-5–C-1	53	55	66	57	64	58	63
C-5–O-5–C-1–C-2	−57	−57	−60	−55	−60	−61	−62
O-5–C-1–C-2–C-3	57	53	59	56	52	57	55
C-4–C-5–C-6–O-6	52	61	54	59	54	51	56
O-5–C-5–C-6–O-6	−70	−54	−65	−60	−65	−66	−54
O-2–C-2–C-3–O-3	57	63	64	64	54	59	55
O-2–C-2–C-3–C-4	177	183	179	179	177	178	178
O-2–C-2–C-1–O-5	183	172	178	182	180	181	183
O-3–C-3–C-4–O-4	−70	−63	−71	−70	−64	−71	−67
O-3–C-3–C-4–C-5	173	179	175	177	180	178	181
O-3–C-3–C-2–C-1	185	187	185	187	184	185	183
O-2–C-2–C-1–O -4″	64	50	59	65	61	53	62
O-5–C-1–O′-4–C -4	115	114	115	107	119	105	114
C-2–C-1–O′-4–C′-4	−125	−117	−127	−136	−120	−126	−126
C-1–O -4–C′-4–C -3	124	129	129	130	130	130	126
C-1–O′-4–C′-4–C′-5	−119	−119	−111	−119	−114	−115	−119

"The primed numbers refer to atoms on neighboring residues

TABLE V

INTRAMOLECULAR, HYDROGEN-BOND LENGTHS AND ANGLES

Hydrogen bond	*Distance (Å)*	*Hydrogen bond*	*Angle (deg)*
O-12 O-73	2 81	C-12–O-12 O-73	115
O-22 O-13	2 86	C-22–O-22 O-13	115
O-32 O-23	2 77	C-32–O-32 O-23	119
O-42 O-33	2 86	C-42–O-42 O-33	119
O-52 O-43	2 80	C-52–O-52 O-43	115
O-62 O-53	2 82	C-62–O-62 O-53	117
O-72 O-63	2 82	C-72–O-72 O-68	117
		C-13–O-13 O-22	115
		C-23–O-23 O-32	115
		C-33–O-33 O-42	114
		C-43–O-43 O-52	118
		C-53–O-53 O-62	117
		C-63–O-63 O-72	114
		C-73–O-73 O-12	116

TABLE VI

INTERMOLECULAR, HYDROGEN-BOND LENGTHS AND ANGLES

Hydrogen bonds involving primary and secondary hydroxyl groups				*Hydrogen bonds involving water molecules*					
Bond		*Distance (Å)*	*Angle (deg.)*	*Bond*			*Distances (Å)*		*Angle (deg.)*
C-12–O-12	O-23	3.09	116	O-W3	O-W1	O-36	2.89	2.75	106
C-12–O-12	O-62	2.74	107	O-66	O-W2	O-W3	2.83	2.78	103
C-13–O-13	O-23	2.80	119	O-66	O-W2	O-W7	2.83	2.87	116
C-22–O-22	O-13	3.14	115	O-W3	O-W2	O-W7	2.78	2.87	101
C-23–O-23	O-13	2.80	119	O-16	O-W3	O-W1	2.84	2.89	105
C-32–O-32	O-73	3.24	114	O-16	O-W3	O-W2	2.84	2.78	124
C-33–O-33	O-73	2.87	116	O-16	O-W3	O-46	2.84	2.78	113
C-42–O-42	O-63	3.05	111	O-W1	O-W3	O-W2	2.89	2.78	104
C-43–O-43	O-63	2.81	116	O-W1	O-W3	O-46	2.89	2.78	111
C-53–O-53	O-53	2.86	119	O-W2	O-W3	O-46	2.78	2.78	99
C-62–O-62	O-12	2.74	112	O-22	O-W4	O-63	2.82	2.69	126
C-62–O-62	O-43	3.04	115	O-26	O-W5	O-56	2.65	3.07	159
C-63–O-63	O-43	2.81	117	O-32	O-W6	O-53	2.57	2.93	120
C-73–O-73	O-33	3.11	117	O-32	O-W6	O-49	2.57	2.36	119
C-73–O-73	O-33	2.87	118	O-76	O-W7	O-36	2.97	2.75	95
C-16–O-16	O-46	2.87	112	O-76	O-W7	O-W2	2.97	2.87	128
C-26–O-26	O-46	2.75	113	O-36	O-W7	O-W2	2.75	2.87	108
C-46–O-46	O-16	2.87	131	O-26	O-W8	O-76	2.68	2.52	115
C-46–O-46	O-26	2.75	102	O-52	O-W9	O-73	2.84	2.76	118
				O-52	O-W9	O-W6	2.84	2.36	109
				O-73	O-W9	O-W6	2.76	2.36	74

dependent data-sets. In the case of the 2,5-diiodobenzoic acid complex there was a torus of heavy electron density at each end of the cavity, parallel to each other and normal to the seven-fold axis of cycloheptaamylose (Fig. 5). One torus was at the level of O-2 and O-3 and the other at the C-6 level. Only a single such torus, at the secondary-hydroxyl end of cycloheptaamylose, was observed in the difference map of the *m*-iodobenzoic acid, *m*-bromobenzoic acid, *m*-iodophenol, and 2-bromo-5-*tert*-butylphenol complexes. As expected, no such prominent density was observed with the *m*-toluic acid complex. This clearly identified the common iodine atom between the diiodo- and monoiodo-benzoic acids. Thus, simple model-building with the two iodine atoms of diiodobenzoic acid occupying a site on each torus of density (Fig. 5) showed the carboxylic acid group of the guest molecule to be near the primary-hydroxyl end of the cycloheptaamylose molecule. The carboxyl group of the guest molecule is not in a position to accept any hydrogen bonds with the primary hydroxyl groups. This was also indicated by the observation that all of the C-6–O-6 bonds are in *gauche–gauche* disposition, meaning that the primary hydroxyl groups point away from the center of the cycloheptaamylose (Fig. 4). It is obvious that a primary hydroxyl group would have to point inwards if it were hydrogen-bonded to the guest

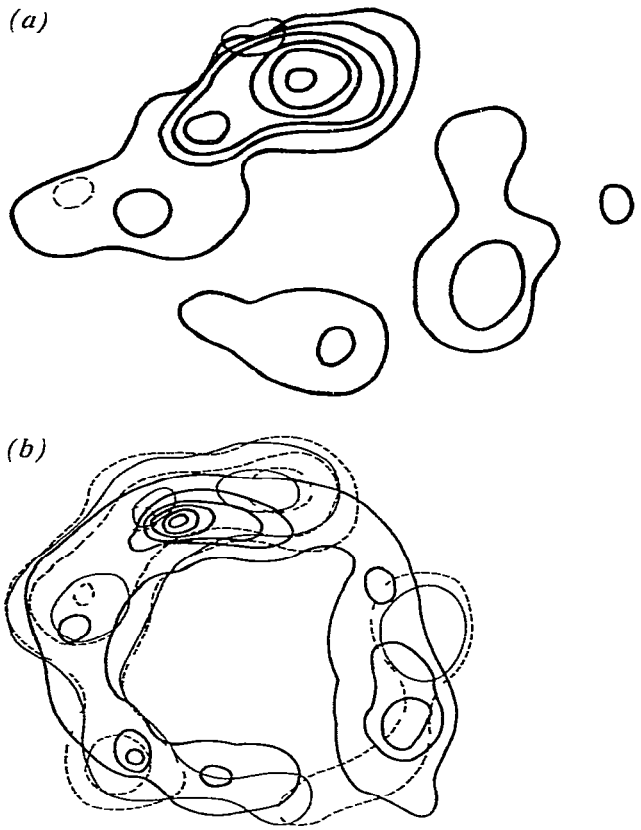


Fig 5 Sections (*a*) $Z = 5{\cdot}48$ and (*b*) $Z = 22{\cdot}48$ of the difference electron-density map showing the disordered heavy-atom sites The heavy continuous lines light continuous lines and dashed lines correspond to 2 5-diiodobenzoic acid *m*-bromobenzoic acid, and *m*-iodobenzoic acid respectively

molecule, as was observed for cyclohexaamylose[10] The positions for the iodine atoms of the disordered guest would place the carboxylic acid group near the level of the primary-hydroxyl oxygen atoms, allowing, but not proving, that the only possible hydrogen bond between guest and host is that of the carboxylic acid hydrogen to a primary-hydroxyl oxygen atom

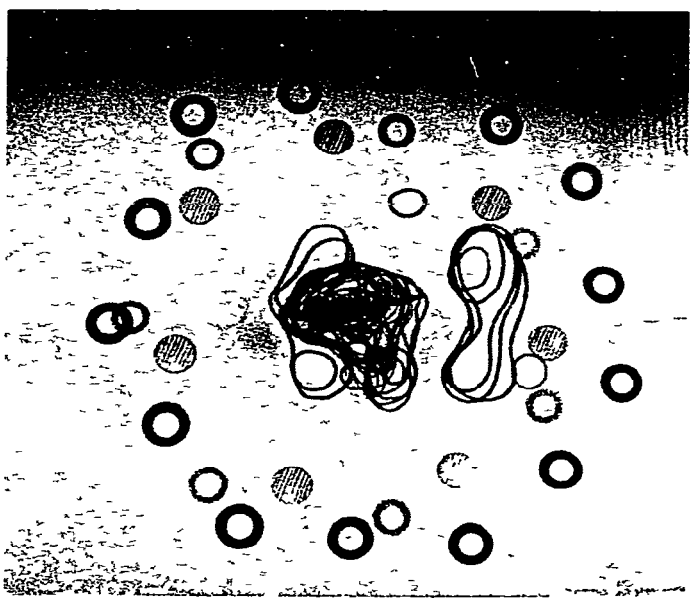
Disorder — In spite of the extensive disorder of the guest molecules, some analysis can be made of the heavy-atom peaks in the two torus sections of the 2,5-diiodobenzoic acid complex It should be noted that the two tori are 5 9 Å apart The torus at the secondary-hydroxyl end is common to five heavy-atom-containing guests The electron densities of the three benzoic acid difference maps were superimposed, and the section containing the torus at the primary and secondary ends are shown in Figs 5a and b. respectively It is relatively easy to observe seven sites in the torus at the secondary end The torus on the primary end is present only for the 2,5-diiodobenzoic acid guest and is somewhat less regular Several of the distances between the four most prominent peaks of the torus at the secondary end and those of the

primary end are 6 8–7 5 Å, which are reasonably close to that expected in 2,5-diiodobenzoic acid (7 1 Å) However, many of the distances involving the prominent peaks at the primary and secondary ends, and their two-fold related counterparts, are considerably less than the iodine–iodine contact-distance (4 3 Å), providing a basis for the disorder of the guest molecules It is obvious from the peaks on the two tori that the plane of the diiodobenzoic acid cannot be parallel to the seven-fold axis of the cycloheptaamylose, the amount of tilt may be estimated to be in the range 20–30°

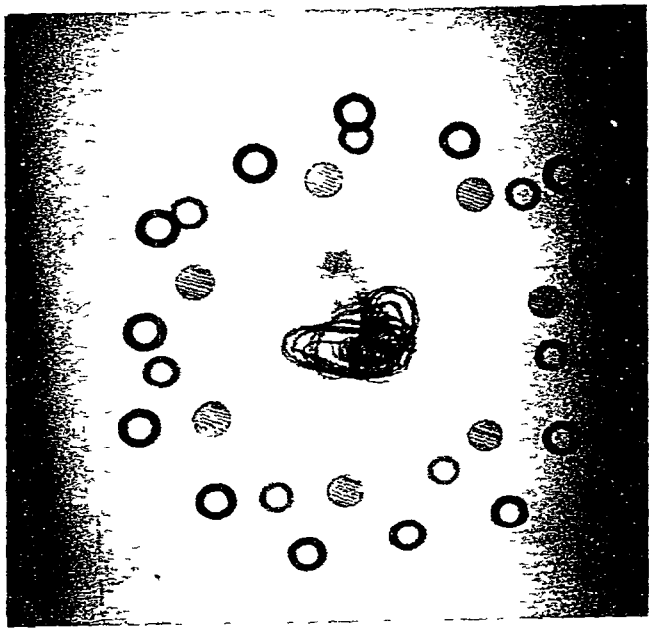
It is very common for monocarboxylic acids to associate in non-aqueous environments, comparable to the hydrophobic interior of the cyclohepta-amylose channel in the crystal, by forming the doubly hydrogen-bonded dimer For such a dimer of the 2,5-diiodobenzoic acid guest, the closest iodine–iodine distance would be 6 2 Å across the two-fold axis relating the two monomer residues Since, as discussed previously, the carboxylic acid of the guest was shown to be at the primary end, distances between peaks of the torus at the primary end and those of its counterpart generated by the nearest two-fold axis were calculated These distances ranged from 4 1 to 6 2 Å, with the high-occupancy iodine sites in the lower end of the range, and usually far less than the expected 6 2 Å for dimer formation This result eliminates the possibility of the hydrogen-bonded dimer as the predominant conformation of the guests

There are distinct differences between the electron densities inside the cavity for the phenol complexes (Fig 6), all of which have been shown by chemical analysis to be 1 1 complexes The electron density of the *m*-iodophenol complex shows considerable similarity to that of *m*-iodobenzoic acid The replacement of hydroxyl for carboxyl evidently has little effect on the position of binding of the guest On the

(a)



(b)



(c)

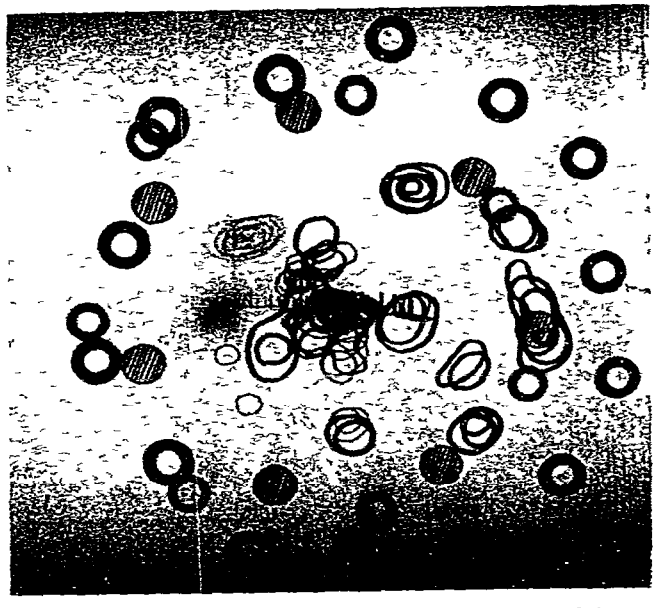


Fig 6 Photographs of the electron density in the cavity of the cycloheptaamylose corresponding to (*a*) 2-bromo-4-*tert*-butylphenol, (*b*) 2-bromo-5-*tert*-butylphenol, and (*c*) *m*-iodophenol The outline of the cycloheptaamylose is achieved by drawing circles corresponding to the positions of the oxygen atoms Open circles represent the primary and secondary hydroxyl-group oxygen atoms and the cross-hatched circles represent the glycosidic oxygen atoms

other hand, the electron density of the 2-bromo-4-*tert*-butylphenol complex also shows some of the sites of the torus at the secondary end of the cavity This requires the *tert*-butyl group to be at the primary end and the bromo and hydroxyl groups at the secondary The electron density of the 2-bromo-5-*tert*-butylphenol complex shows no peaks at the site of either torus Evidently this complex has a different kind of disorder, possibly an up–down rather than rotational disorder

The usual convention for describing the position of the guest has 'normal orientation' with the most polar group at the secondary end of the cavity and 'reverse orientation' with the most polar group at the primary end Therefore, we classify the 2 5-diiodobenzoic acid, *m*-bromobenzoic acid *m*-iodobenzoic acid, *m*-iodophenol, and probably the *m*-toluic acid guests as having reverse orientation, whereas the 2-bromo-4-*tert*-butylphenol guest has normal orientation and the 2-bromo-5-*tert*-butylphenol guest possibly has both We conclude that, energetically, there is little to choose between normal and reverse orientation in the cycloheptaamylose cavity for this type of guest

In most early binding-studies in solution, it was concluded that meta-substituted guests enter the cavity from the secondary-hydroxyl end, with the more hydrophobic end of the guest going deeper and the more hydrophilic end remaining at the surface of the secondary end in close contact with solution water[3] However Bergeron conducted n m r studies on the binding of a series of carboxylic acids to cyclohexaamylose[19] The guests were chosen so that only one-to-one complexes would result He found that benzoic acid itself binds in the cyclohexaamylose cavity 87 times more tightly than the corresponding benzoate anion and that the carboxylic acid group was down in the cavity at the primary-hydroxyl end He also concluded from his study that solvation of the polar groups was the most important factor in the positioning and stability of the guest in the cyclohexaamylose cavity In our crystal structure, contact of the guest with water through the secondary end is prohibited by the formation of the cycloheptaamylose dimer Thus, in solution the wider (secondary) end offers most contact with water than the narrower (primary) end whereas the reverse is true in our crystal structure Therefore, we might indeed expect to find the hydrophilic group of the guest at the primary hydroxyl end in all cases

Straub and Bender[20] investigated the aqueous decarboxylation of benzoylacetic acids in the presence of cyclohexa- and cyclohepta-amyloses Decarboxylation is accelerated by cycloheptaamylose but inhibited by cyclohexaamylose They concluded that this inhibition arises from a conformational constraint on the included acid, resulting in non-productive binding Although the benzoic acid derivatives used here resemble products rather than substrates, presumably the dimerization of cyclohepta-amylose would similarly dominate the orientation of analogous substrates and thus lead to non-productive binding

Crystal studies on cyclohexaamylose with a number of phenyl-substituted guests also exhibit a structural component that results in non-productive binding[10 11] In all of these crystal structures, the polar group of the guest molecule, namely the carboxyl group in the phenyl esters and the hydroxyl group in the phenols, does not

form any hydrogen bonds with the secondary hydroxyl groups of the cycloamylose as would be expected from the functional mechanism In fact, the polar groups are usually found near the primary-hydroxyl side of the cycloamylose In cyclohexaamylose complexes, the groups attached to the phenyl ring are often hydrogen bonded to symmetry-related cyclohexaamylose molecules in the unit cell[11], bonds which, if present in solution, would be furnished by water molecules Indeed, the hydroxyl groups of all of the cycloamylose structures in crystals thus far investigated are extensively hydrogen bonded to the symmetry-related cycloamylose molecules[10 11] The strength of the intermolecular interaction through the hydroxyl groups is revealed by the observation that the crystal structures of the uncomplexed cyclohexa- and cycloheptа-amyloses are isomorphous to at least one complex crystal form, and indeed were solved by means of comparison with the isomorphous complex The hydrogen bonding between cycloamylose molecules appears to be a dominating force in the crystal structures and prohibits the interaction expected between the guests and the secondary hydroxyl groups

The concept of non-productive binding and the isomorphism between complexed and uncomplexed cycloamylose crystal structures does not support the induced-fit model proposed by Saenger and co-workers[26] Their model is based on their X-ray structure determination of the uncomplexed cyclohexaamylose They have described this conformation as being in strain because one of the glucosyl residues is rotated toward the cavity, whereas the other five retain nearly six-fold symmetry It is not clear why this rotation should be a function of a lack of guest, because this structure is isomorphous to that of the cyclohexaamylose–hydrogen iodide complex from which it was solved[26] The induced-fit theory is further weakened by the finding that uncomplexed cycloheptaamylose in the crystal structure shows little deviation from seven-fold symmetry

We conclude that the only important factor that determines strength and direction of binding in the crystal structures investigated is the hydrophobic interaction of the benzene ring and its substituents, subject to the constraint of dimer formation by the secondary-hydroxyl ends of the cycloheptaamylose molecules

Cycloheptaamylose dimer — It would seem to us that the function of the head-to-head dimer of cycloheptaamylose in the crystal form is similar to that of the capped derivatives already described[5 6] dimerization restricts the access of water at the secondary end and thereby influences the orientation and stability of the guest The dimer formation also brings the secondary ends very close together and restricts, to the point of overcrowding the guests, the amount of room available Complexes involving two molecules of cycloamylose and one or two molecules of guest are being found with increasing frequency in solution studies Guests include Methyl Orange[27 28], Orange I[29], 6-*p*-toluidinonaphthalene-2-sulfonate[30], heptane[31], cyclohexane[31], 4-biphenyl carboxylate anion[21], and *p*-methylcinnamate anion[32]

Crystal structures of cyclohepta-amylose with 1-propanol[33], *p*-iodophenol[34], and *p*-nitroacetanilide[35] have recently been reported Although they crystallize in different space-groups, these structures have the head-to-head dimer formation and

crystal packing very similar to that of the structures reported here Head-to-head and also tail-to-tail contacts in the complex of cyclooctaamylose with 1-propanol have recently been announced[36] Functionally, the head-to-head dimerization restricts the access of water to the secondary end of the cycloamylose in the crystal and thereby influences the orientation and stability of the guest

Water molecules — The water molecules were readily located during the structure refinement Comparison of the temperature parameters of the water molecules in the phenol complexes (Table II) with those of benzoic acid complexes showed the former to be considerably higher This result shows that, despite the fact that most sites are in common, the water molecules are less tightly bound in the phenol complexes Indeed, there was a noticeable difference in the rate of drying-out of the crystals of these complexes when left exposed to air Crystals of the 2,5-diiodobenzoic acid complex in fact gave a good diffraction pattern after drying whereas those of the 2-bromo-4-*tert*-butylphenol complex did not

There is no involvement of water molecules with the interior of the cavity The liberation of unfavorably bound (strained) water from the cavity has been cited as one of the driving forces for complex-formation[26] As expected, increasing amounts of cavity water were found in the crystal structures of uncomplexed cyclohexaamylose[26], cycloheptaamylose[37], and cyclooctaamylose[38], having respectively two water molecules, approximately six water molecules in eight sites, and an unspecified number of water molecules in twelve sites inside the cavity This trend might well be reflected in the thermodynamics of binding in solution

CONCLUSIONS

The most important result from this crystallographic investigation is the head-to-head dimer formation by the cycloheptaamylose molecules This feature dominates the orientation of the guest molecules in the cavity and acts rather like the capping ' achieved by chemical modification Two consequences of the capping by dimer formation are the exclusion of water contacts at the secondary-hydroxyl end of the cycloheptaamylose, and the creation of overcrowding conditions for the guests Neither condition would be expected to be present in solution

The well-ordered, tightly bound, water molecules between the cycloheptaamylose dimers are a prominent feature of the crystal structure, and are responsible for holding the structure together The guests are always highly disordered, and are found in positions that are reasonable for products or inhibitors of catalysis by cycloheptaamylose

ACKNOWLEDGMENTS

X-Ray 72, a system of crystallographic computer programs created by Dr J Stewart, Computer Science Center, University of Maryland, was used for most calculations For time on the CDC-6600 computer, we thank the Computing Center,

Indiana University–Purdue University at Indianapolis The crystallographic facilities were supported by Heart Center grant HE-06307 The research was supported by National Science Foundation grant PCM 74 22992 and National Institutes of Health grant GM-24623 We are indebted to Dr R L Van Etten, Department of Chemistry, Purdue University for crystals of the cycloheptaamylose complexes and for valuable discussions

REFERENCES

1 N NAKAMURA AND K HORIKOSHI, *Agric Biol Chem (Tokyo)*, 40 (1976) 935–941
2 M NOLTMEYER AND W SAENGER, *Nature*, 259 (1976) 629–632
3 M L BENDER AND M KOMIYAMA, *Cyclodextrin Chemistry* Springer–Verlag, New York, 1978
4 M L BENDER, R L VAN ETTEN, AND G A CLOWES, *J Am Chem Soc*, 88 (1966) 2318–2319
5 J EMERT AND R BRESLOW, *J Am Chem Soc*, 97 (1975) 670–672
6 I TABUSHI, K SHIMOKAWA, N SHIMIZU, H SHIRAKATA, AND K FUJITA, *J Am Chem Soc*, 98 (1976) 7855–7856
7 Y KITARA AND M BENDER, *Bioorg Chem*, 4 (1975) 237–249
8 R L VAN ETTEN, J F SEBASTIAN, G A CLOWES, AND M L BENDER, *J Am Chem Soc*, 89 (1967) 3242–3252
9 A HYBL, R E RUNDLE, AND D E WILLIAMS, *J Am Chem Soc*, 87 (1965) 2779–2788
10 W SAENGER, *Environmental Effects on Molecular Structure and Properties*, B PULLMAN (2nd ed), D. Reidel Publishing Company, Dordrecht–Hellar, 1976, p 265
11 K HARATA, *Carbohydr Res*, 42 (1976) 265–270
12 J A HAMILTON, M N SABESAN, L K STEINRAUF, AND A GEDDES, *Biochem Biophys Res Commun*, 73 (1976) 659–664
13 J A HAMILTON, L K STEINRAUF, AND R L VAN ETTEN, *Acta Crystallogr, Sect B*, 24 (1968) 1560–1562
14 J F CUTFIELD, S M CUTFIELD, G G DODSON, E J DODSON, AND M N SABESAN, *J Mol Biol*, 87 (1974) 23–30
15 M G ROSSMANN AND D M BLOW, *Acta Crystallogr*, 15 (1962) 24–31
16 P TOLLIN, P MAIN, AND M G ROSSMANN, *Acta Crystallogr*, 20 (1966) 404–407
17 *International Tables for X-Ray Crystallography*, Vol III, Kynoch Press, Birmingham, 1962, pp 202, 214
18 B HINGARTY AND W SAENGER, *J Am Chem Soc*, 98 (1976) 3357–3365
19 R J BERGERON, M A CHANNING, K A MCGOVERN, AND W P ROBERTS, *Bioorganic Chem*, 8 (1979) 263–281
20 T S STRAUB AND M L BENDER, *J Am Chem Soc*, 94 (1972) 8881–8888
21 R I GELB, L M SCHWARTZ, C T MURRAY, AND D A LAUFER, *J Am Chem Soc*, 100 (1978) 3553–3559
22 R J BERGERON AND M A CHANNING, *J Am Chem Soc*, 101 (1979) 2511–2516
23 R I GELB L M SCHWARTZ, R F JOHNSON AND D A LAUFER *J Am Chem Soc* 101 (1979) 1869–1979
24 R BERGERON AND R ROWAN III, *Bioorg. Chem*, 5 (1976) 425–436
25 G KITAURA AND M L BENDER, *Bioorg Chem*, 4 (1975) 237–249
26 P C MANOR AND W SAENGER, *J Am Chem Soc*, 96 (1974) 3630–3639
27 N H HENNRICH AND F CRAMER, *J Am Chem Soc*, 87 (1965) 1121–1126
28 K HARATA, *Bull Chem Soc Jpn*, 49 (1976) 1493–1501
29 A HARADA, M FURUE, AND S NOZAKURA, *Macromolecules*, 9 (1976) 701–704
30 H KONDO, H NAKATANI, AND K HIROMI, *J Biochem (Tokyo)*, 79 (1976) 393–405
31 A WISHNIA AND S J LAPPI, *J Mol Biol*, 82 (1974) 77–89
32 R I GELB, L M SCHWARTZ, AND D A LAUFER, *J Am Chem Soc*, 100 (1978) 5875–5879
33 J J. STEZOWSKI K H JOGUN, E ECKLE, AND K BARTELS, *Nature*, 274 (1978) 617–619
34 K H JOGUN AND J J STEZOWSKI, *Nature*, 278 (1979) 667–668

35 M M HARDING, J M MACLENNAN, AND R M PATON, *Nature*, 274 (1978) 621–623
36 K LINDNER AND W SAENGER, *Biophys Biochem Res Commun*, 92 (1980) 933–938
37 K LINDNER AND W SAENGER, *Angew Chem Int Ed Engl* 17 (1978) 694–695
38 J M MACLENNAN AND J J STEZOWSKI, *Biochem Biophys Res Commun* 92 (1980) 926–932