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THE CRYSTAL AND MOLECULAR STRUCTURE OF TRI-*O*-ETHYLAMYLOSE (TEA 3)*

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ABSTRACT

The crystal and molecular structure of a tri-*O*-ethylamylose polymorph, TEA 3, has been solved by stereochemical conformation and packing analysis, combined with X-ray fibre diffraction analysis. The unit cell is orthorhombic, space group $P2_12_12_1$, with a = 15.36 (±0.03) Å, b = 12.18 (±0.05) Å, and c (fibre repeat) = 15.48 (±0.01) Å. The actual chain conformation is a 4_3 helix with the EtO-6 group in the *tg* position, as was found in the polymorph TEA 1.

INTRODUCTION

The conformation and crystalline packing of tri-*O*-ethylamylose[1] (TEA 1) and of tri-*O*-ethylamylose complexed with various small molecules, for example, nitromethane (TEA 1-N), chloroform (TEA 1-C2), and dichloromethane (TEA 1-DCM2), have been recently determined[2]. An additional polymorph, termed TEA 3, occurs when these tri-*O*-ethylamylose complexes are dried. As several other polymorphs of tri-*O*-ethylamylose, both with and without complexing molecules in their crystal structures, are known, it is of interest to determine as many of these structures as possible. The information thus obtained may lead to further insight into the processes of solvation and transformation occurring in polymer crystals. We now report on the structure of the polymorph TEA 3.

EXPERIMENTAL

Tri-*O*-ethylamylose (TEA) was prepared as previously described[1]. Films were cast from solutions in 1,4-dioxane and stretched ~300% in glycerol at 130°. The stretched films were then annealed *in vacuo* at 235° for 20 min. X-Ray fibre diagrams obtained on these films revealed the TEA 1 polymorph. When the fibres of TEA 1 were sealed in beryllium-glass capillaries over a 1:5 mixture of chloroform (solvent) to ethanol (non-solvent), they were converted into the tri-*O*-ethylamylose–chloroform

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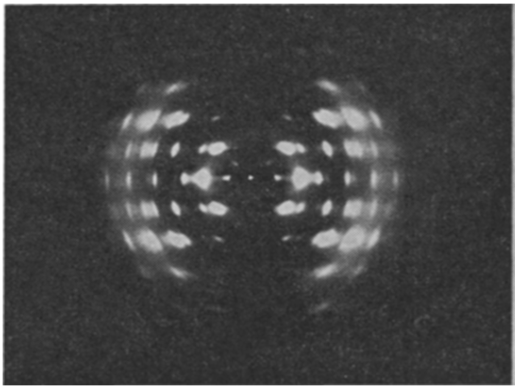
*Conformation and Packing Analysis of Polysaccharides and Derivatives: Part V.



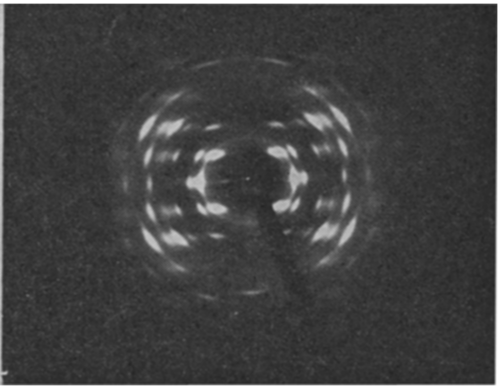


Fig. 1 (left) Fibre diffraction diagram of TEA 1-C2 taken in a cylindrical camera with radius 5.73 cm.

Fig. 2 (right). Fibre diffraction diagram of TEA 3 taken in a cylindrical camera with radius 5.73 cm.

complex (TEA 1-C2). A representative diffractogram of the TEA 1-C2 complex is shown in Fig. 1. Opening of the capillaries and removal of the solvent–non-solvent mixture *in vacuo* resulted in the new modification (TEA 3). A typical X-ray diagram of TEA 3 is reproduced in Fig. 2.

The *d*-spacings were measured on diffraction diagrams recorded with CuKα radiation on flat films. The diffracted intensities were measured on diagrams recorded in an evacuated, cylindrical camera on multiple film packs of Ilford Industrial-G X-ray film. The intensities thus collected were corrected and reduced to structure amplitudes as previously described[1]. Unobserved intensities were assigned uncorrected relative-intensity values of one-half the minimum observable value in the corresponding region of the scattering angle.

The unit cell, as determined by least-squares refinement, was orthorhombic with dimensions $a = 15.36\ (\pm\ 0.03)$ Å, $b = 12.18\ (\pm\ 0.05)$ Å, and c (fibre repeat) $= 15.48\ (\pm\ 0.01)$ Å. The only meridional reflection observed was the fourth order with a *d*-spacing of 3.87 Å, as in TEA 1[1]. Systematic absences of reflections indicated $P2_12_12_1$ as the space group.

RESULTS AND DISCUSSION

In view of the similarities between TEA 1 and TEA 3, *i.e.*, the same fibre repeat and the presence of the fourth-order meridional reflection, packing refinement was performed with the conformation of TEA 1 as the initial model, using methods previously described[3]. The unit cell is similar in volume to that of TEA 1, indicating that sections of two polymer chains pass through the unit cell. The stereochemical refinement resulted in a good packing model with no unreasonably short intermolecular contacts.

The best packing model was then refined[3] against the observed X-ray structure amplitudes. The final, crystallographic *R* factor, including all unobserved reflections, was 0.29 and was calculated from the equation:

$$R = \sum||F_o| - |F_c||/\sum|F_o|,$$

where $|F_o|$ are the observed and $|F_c|$ the calculated structure amplitudes. The R factor including only the observed structure amplitudes was 0.22. The refinement of the best packing model against the structure amplitudes produced only insignificant changes in the model.

Final co-ordinates for one residue are reported in Table I. A representation of one residue of TEA, showing atom labelling, is given in Fig. 3. The residue is in the standard position when the vector pointing from the origin to O-4 (glycosidic oxygen) is along the $-y$ axis. Rotation of this vector about the z axis of the co-ordinate system and its translation along the z axis produce the helix rotation and translation. The co-ordinates reported in Table I have been transformed to the final helix rotational and translational position, which corresponds to a rotation of 27.7° and a shift of 0.177 Å. Bond lengths, bond angles, and torsion angles of one residue are given in Table II. The glycosidic bond angle is 122.1° and deviates insignificantly from that for TEA 1, and the optimal virtual-bond length is again 4.35 Å. The rotational position of EtO-6 is —37° from the exact *tg*[*] position, as compared with —35° from the *tg* position in the best stereochemical model of TEA 1. An isotropic temperature factor of 3.0 was used throughout the refinement. The calculated and observed

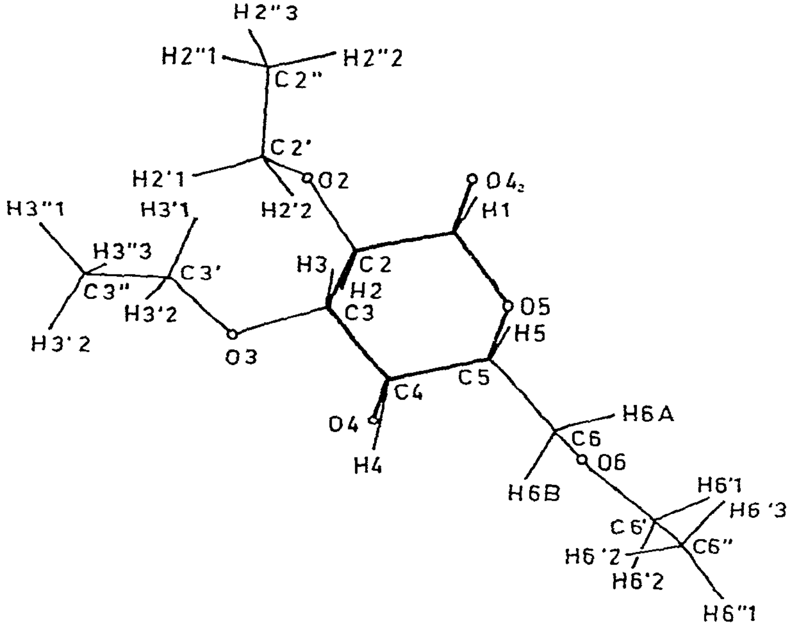


Fig. 3. Representation of one residue of tri-*O*-ethylamylose, showing atom labelling

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[*] For a description of *tg*, see Ref. 1.

TABLE I

CARTESIAN CO-ORDINATES (Å) OF FIRST RESIDUE[a] OF TEA 3 (VIRTUAL-BOND LENGTH = 4.35 Å)

Atom	x	y	z	*Atom*	x	y	z
O-4	4.493	−1.243	0.177	H-3	4.363	−2.072	2.534
C-1	1.734	−1.403	3.207	H-4	2.653	−2.191	0.106
C-2	2.378	−2.742	2.858	H-5	3.186	0.250	1.730
C-3	3.601	−2.528	1.973	H-6A	1.081	0.887	0.305
C-4	3.255	−1.650	0.775	H-6B	1.800	−0.154	−0.774
C-5	2.525	−0.370	1.199	H-2′1	2.155	−5.338	4.372
C-6	2.008	0.468	0.046	H-2′2	0.974	−4.401	3.749
O-2	2.715	−3.415	4.067	H-3′1	4.278	−4.794	3.237
O-3	4.067	−3.788	1.485	H-3′2	5.659	−3.937	2.737
O-4$_2$	2.597	−0.653	4.047	H-6′1	1.336	2.394	−1.281
O-5	1.404	−0.702	2.035	H-6′2	2.626	2.071	−2.285
O-6	2.907	1.509	−0.359	H-2″1	1.464	−4.991	6.457
C-2′	1.748	−4.374	4.458	H-2″2	0.111	−4.184	5.778
C-2″	1.159	−4.196	5.842	H-2″3	1.493	−3.289	6.250
C-3′	4.860	−4.531	2.403	H-3″1	6.403	−5.911	2.023
C-3″	5.397	−5.778	1.752	H-3″2	4.837	−6.608	2.068
C-6′	2.385	2.409	−1.320	H-3″3	5.324	−5.689	0.709
C-6″	2.882	3.846	−1.142	H-6″1	3.100	4.259	−2.083
H-1	0.864	−1.569	3.772	H-6″2	3.750	3.846	−0.551
H-2	1.682	−3.345	2.353	H-6″3	2.139	4.419	−0.672

[a] The residue has been shifted 1/4 in *a* as required for space group $P2_12_12_1$, rotated 27.7°, and translated 0.177 Å along the *z* axis as compared with the standard position of O-4 as $(0,-y_0,0)$

structure amplitudes are reported in Table III, and the shortest inter- and intramolecular contact-distances are listed in Table IV.

CONCLUSION

With the knowledge of the crystal and molecular structure of TEA 3, we are now able to detect changes in the unit cell and packing that occur during the transition TEA 1→TEA 1-C2→TEA 3. The unit cell is orthorhombic and the space group is $P2_12_12_1$ in all cases, despite the fact that $a = b = 14.7$ Å for TEA 1-C2. The fibre repeat remains at $c = 15.48$ Å for all three structures, with only a fourth-order meridional reflection appearing in all X-ray diagrams. Therefore, we conclude that the molecular conformation is a four-fold left-handed (4_3) helix with a 3.87-Å rise per residue. A right-handed helix is not likely, due to the presence of excessively short intramolecular contacts. The base plane differs somewhat in the three crystal structures: for TEA 1, $a = 16.13$ Å and $b = 11.66$ Å, resulting in a base-plane area of 188.1 Å^2; for TEA 3, with $a = 15.36$ Å and $b = 12.18$ Å, the area is 187.1 Å^2; for TEA 1-C2, it is 216.1 Å^2. The inter-chain distances between corner and centre chains are 9.95 Å for TEA 1, 9.80 Å for TEA 3, and 10.4 Å for TEA 1-C2. The d_{110} spacings are 9.45, 9.54, and 10.4 Å, respectively. It is therefore evident that the packings of

TABLE II

BOND LENGTHS, BOND ANGLES, AND TORSION ANGLES FOR ONE RESIDUE[a]

Bond lengths (Å)		*Bond angles (degrees)*	
O-4–C-4	1.434 (8)	O-4–C-4–C-3	107.2 (1.7)[b]
C-4–C-3	1.525 (2)	O-4–C-4–C-5	106.8 (−1.8)
C-4–C-5	1.534 (9)	C-3–C-4–C-5	111.8 (1.5)
C-1–C-2	1.526 (3)	C-4–C-3–C-2	110.8 (0.3)
C-1–O-4$_2$	1.418 (3)	C-3–C-2–C-1	110.4 (−0.1)
C-3–C-2	1.515 (−6)	C-4–C-5–O-5	109.8 (−0 2)
C-5–O-5	1.439 (3)	C-5–O-5–C-1	114.7 (0.7)
C-2–O-2	1 424 (1)	C-2–C-1–O-5	110.2 (1.0)
C-3–O-3	1.430 (1)	C-2–C-1–O-4$_2$	110 0 (1.6)
C-5–C-6	1.517 (3)	O-5–C-1–O-4$_2$	111.9 (0 3)
C-6–O-6	1.434 (7)	C-3–C-2–O-2	111.7 (0 9)
C-1–O-5	1.405 (−9)	C-1–C-2–O-2	108 7 (−0 6)
		C-4–C-3–O-3	109 6 (−0 1)
Torsion angles (degrees)		C-2–C-3–O-3	108 3 (−1.3)
O-5–C-1–C-2–C-3	56 4 (0 4)	C-4–C-5–C-6	114.4 (1.7)
C-1–C-2–C-3–C-4	−51.6 (1.6)	O-5–C-5–C-6	107.7 (0 8)
C-2–C-3–C-4–C-5	50.1 (−2 9)	C-5–C-6–O-6	113.7 (1.9)
C-3–C-4–C-5–O-5	−51 9 (3.5)	C-1–O-4$_2$–C-4$_2$	122.1
C-4–C-5–O-5–C-1	59.1 (−2 0)		
C-5–O-5–C-1–C-2	−61.7 (0 5)		
O-4–C-4–C-5–O-5	−168 9		
O-4–C-4–C-3–C-2	166 9		
O-4$_2$–C-1–C-2–C-3	−67 5		
O-4$_2$–C-1–O-5–C-5	61 0		
O-5–C-5–C-6–O-6	143 3		
C-4$_2$–O-4$_2$–C-1–H-1	−55 3		
C-1–O-4$_2$–C-4$_2$–H-4$_2$	−41.6		

Ethyl group pendant atoms[c]

Bond lengths (Å)		*Torsion angles (degrees)*	
O-2–C-2′	1.418	C-3–C-2–O-2–C-2′	97.7
C-2′–C-2″	1 514	C-2–O-2–C-2′–C-2″	−123.5
O-3–C-3′	1.422	O-2–C-2′–C-2″–H-2″1	−112.9
C-3′–C-3″	1.506	C-2–C-3–O-3–C-3′	158.7
O-6–C-6′	1.417	C-3–O-3–C-3′–C-3″	−175.0
C-6′–C-6″	1.531	O-3–C-3′–C-3″–H-3″1	137.3
		C-5–C-6–O-6–C-6′	−172.6
Bond angles (degrees)		C-6–O-6–C-6′–C-6″	145 4
C-2–O-2–C-2′	113.1	O-6–C-6′–C-6″–H-6″1	138 6
O-2–C-2′–C-2″	116.0		
C-3–O-3–C-3′	115.0		
O-3–C-3′–C-3″	110.7		
C-6–O-6–C-6′	114 9		
O-6–C-6′–C-6″	113 4		

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[a] Deviations from Arnott and Scott average values[5] are shown in brackets. [b] Deviation from average value for α-D-glucans (see Ref. 3) [c] All hydrogen parameters not listed are fixed at bond distances of 1.05 Å and at tetrahedral bond angles

TABLE III

OBSERVED AND CALCULATED STRUCTURE AMPLITUDES

hkl	$\|F_{obs}\|$	$\|F_{calc}\|$	*hkl*	$\|F_{obs}\|$	$\|F_{calc}\|$
100[b]	0	0	212	66	70
010[b]	0	0	022[a]	14	4
110	75	63	122	38	57
200	31	22	302[a]	15	4
210[a]	11	0	222,312	81	103
020[a]	11	5	032[a]	18	12
120[a]	12	0	132[a]	18	20
300[b]	0	0	322,402	36	35
220,310	46	35	412,232	29	57
030[b]	0	0			
320[a]	18	14	103	8	3
130[a]	18	1	013[a]	6	1
410,400	42	31	113	14	27
230[a]	20	7	203	20	24
420,330	32	36	213,023	42	49
			123	40	29
101	29	33	303[a]	15	20
011	39	34	223,313	35	43
111	41	45	033[a]	19	23
201[a]	9	7	133,323	29	36
211	32	33			
021	21	36	104[a]	4	4
121	25	44	014[a]	4	9
301	20	26	114	40	45
221,311	55	58	204[a]	11	20
031[a]	15	2	024[a]	13	9
321,401, 131	46	36	214	45	30
			124,304	48	49
411,231	43	56	224,314	39	40
102,012	27	23			
112	11	4			
202	19	6			

[a] Unobserved structure amplitudes taken as one-half the minimum observable $|F|$ in that region of the diffractogram. [b] Systematically absent reflections of space group $P2_12_12_1$.

TEA 1 and TEA 3 are nearly identical*, and that the addition of solvent, which is located in the grooves of the helices, causes only a slight increase in the distance between the antiparallel corner and centre chains. The small increase in density from TEA 1 to TEA 3 is within the experimental error of the measurement.

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*Because of the small difference in TEA 1 and TEA 3, we refer to Figs. 4 and 5 of Ref. 1 for the projections in the *a*,*b*- and $\bar{1}10$-plane.

TABLE IV

SHORTEST ATOM–ATOM CONTACTS

Intermolecular contacts	*Distance (Å)*[a]
C-3″ . . . C-6″[d]	3.46
O-3 . . . H-2′3[d]	2.57
H-6″3 . . . C-3′[d]	2 66
H-6″3 . . . C-3″[d]	2.80
H-3″2 . . . H-3″2[d]	2.14
H-3′2 . . . H-6″3[d]	2 15
H-2″1 . . . H-6A[c]	2.25
H-6″1 . . . H-6″3[c]	2 28
Intramolecular contacts	*Distance (Å)*[a]
O-2 . . . O-4_2[b]	2.76
C-5 . . . O-4_2[b]	2 86
C-3 . . . O-4_2[b]	2.97
C-1 . . . O-3_2	2.97
O-5 . . . C-4_2[b]	3.01
C-6″ . . . O-2[e]	3 31
H-1 . . . O-3_2	2 23
O-5 . . . H-6A[b]	2.37
O-2 . . . H-6″1[e]	2 59
H-5 . . . O-6[b]	2.88
C-1 . . . C-3_2[b]	3.13
H-1 . . . C-4_2[b]	2.68
C-1 . . . H-4_2[b]	2.70
H-3′3 . . . H-2″3[e]	2.19
H-5 . . . H-6A_2	2 62

[a] All distances not due to 1 . . . 4 contacts are greater than the minimum contact distances as set forth in Ref. 4. [b] 1 . . . 4 contacts. Related by symmetry operators: [c] $1/2 + x$, $1/2 - y$, $\bar{z}$; [d] $\bar{x}$, $1/2 + y$, $1/2 - z$. [e] Contacts between first and third residue of the helix.

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REFERENCES

1 T. L. Bluhm, G. Rappenecker, and P. Zugenmaier, *Carbohydr. Res.*, 60 (1978) 241–250.
2 T. L. Bluhm and P. Zugenmaier, *Progr. Colloid Polym. Sci.*, 64 (1978) 132–138.
3 P. Zugenmaier and A. Sarko, *Biopolymers*, 15 (1976) 2121–2139.
4 G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, in G. N. Ramachandran (Ed), *Aspects of Protein Structure*, Academic Press, London and New York, 1963, p. 121–135
5 S. Arnott and W. E. Scott, *J. Chem. Soc., Perkin Trans. 2*, (1972) 324–335.