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DETAILED REFINEMENT OF THE CRYSTAL STRUCTURE OF TRI-*O*-ETHYLAMYLOSE (TEA I)*

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ABSTRACT

The crystal structure of tri-*O*-ethylamylose has been solved by stereochemical conformation and packing analysis and by X-ray fibre diagram analysis. The unit cell is orthorhombic, space group $P2_12_12_1$, with $a = 16.13$ ($\pm$ 0.04) Å, $b = 11.66$ ($\pm$ 0.02) Å, and c (fibre repeat) $= 15.48$ ($\pm$ 0.02) Å. Density measurements, together with the observation of only a fourth-order meridional reflection, indicated that portions of two four-fold helices pass through the unit cell. The actual chain conformation is that of a 4_3 helix with the EtO-6 group in the *tg* (trans to O-5, and gauche to C-4) position. The tri-*O*-ethylamylose structure is compared with those of other amylose derivatives.

INTRODUCTION

The structure of tri-*O*-ethylamylose[1] (TEA) is of interest, first, in comparison with the structures of amylose polymorphs and other amylose derivatives and, second, because changes into different modifications can be studied easily.

The structure of V-amylose consists of an approximate six-fold helix of left-handed chirality packed in an orthorhombic unit-cell, space group $P2_12_12_1$. The exact two-fold screw-axis of the helix was confirmed only recently[2]. Three different rotational positions of HO-6 on three successive residues were established. Thus, the asymmetric unit of the helix consists of three α-D-glucosyl residues. In tri-*O*-acetylamylose (ATA), the chain conformation is a 14_{11} helix, that is, left-handed chirality with 14 residues in 3 complete turns of the helix[3]. The unit cell is orthorhombic with antiparallel chain packing. In tri-*O*-methylamylose (TMA), the conformation of the polymer backbone can be described approximately by a 4_3 helix, but with two different rotational positions of MeO-6 on two successive residues[4]. This again results in a two-fold screw axis along the chain. TMA packs in the orthorhombic space-group $P2_12_12_1$.

The rise per residue of V-amylose (1.32 Å) is very different from that of ATA

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

(3.75 Å) and TMA (3.91 Å). The two amylose derivatives thus far characterized have similar rises per residue, but differ in helix symmetry. Therefore, it was of interest to investigate the structure of TEA in order to determine further the effect of substituent groups on the symmetry of the polysaccharide backbone.

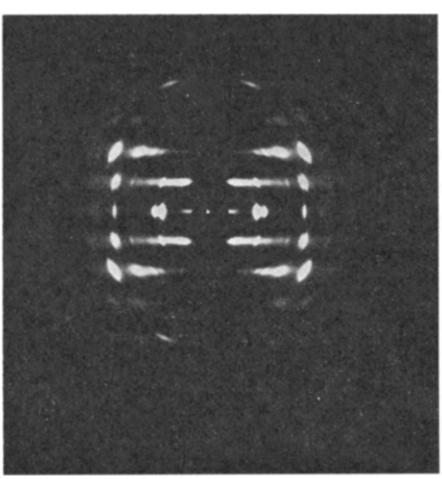
During the course of the investigation, it was observed that various crystalline modifications of TEA can be produced. Some of them incorporate such solvent molecules as chloroform, dichloromethane, and nitromethane in the crystalline lattice. This knowledge served only to increase our interest in the structural investigation.

Changes in crystal modifications due to solvent effects can be easily studied with TEA, and guest molecules located. However, we now report on the most common modification of (TEA 1).

In this investigation, a recently developed method of stereochemical packing analysis was used, which permits simultaneous rotation of substituent groups during both packing analysis and X-ray refinement[2,4]. By this method, the phasing model necessary for refinement against X-ray diffracted intensities was obtained.

EXPERIMENTAL

Tri-*O*-ethylamylose was prepared by homogeneous ethylation of commercially available AVEBE amylose[1]. Films were cast from *p*-dioxane solutions and stretched ~300% in glycerol at 130°. The films were then annealed at 235° for 20 min. The diffracted X-ray intensities were produced by irradiation of the sample with X-rays of the CuKα wavelength, and were collected on cylindrical films (Fig. 1). The optical



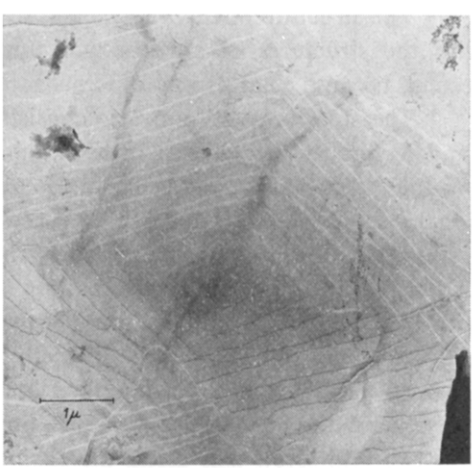


Fig. 1. Fibre diffraction diagram of tri-*O*-ethylamylose taken in a cylindrical camera with radius 5.73 cm.

Fig. 2. Electron micrograph of single crystals of tri-*O*-ethylamylose grown at 160–180° in diethylene glycol.

densities of the diffraction spots were recorded along each layer line with a Joyce–Loebl recording densitometer. The areas of individual peaks, as measured by planimetry, were used as uncorrected relative intensities. For overlapping reflections, which produced peaks with shoulders, the contributions from the individual diffracting planes were separated manually. When this separation was impossible, the structure amplitudes were calculated as composites in the following manner:

$$|F_c| = (\Sigma_i m_i F_{ci}^2)^{0.5},$$

where m_i is the multiplicity, and the summation is carried out over all of the diffracting planes contributing to the composite.

The relative intensities were corrected for Lorentz and polarization factors[5], arcing of reflections, and unequal film-to-sample distances of the diffracted rays. They were then converted into structure amplitudes. Unobserved intensities were assigned uncorrected relative-intensity values of one-half the minimum observable value in that region of the diffractogram.

Single crystals of TEA were grown in diethylene glycol at 160–180° for 20 h, and collected by centrifugation with methanol. Electron transmission photographs were made with a Siemens Elmiskop I electron microscope. A photograph of a typical TEA single crystal is shown in Fig. 2.

The density of TEA fibres, as measured by flotation in carbon tetrachloride–hexane, was 1.16 g.cm^{-3}. The X-ray diffractograms were indexed with an orthorhombic unit-cell, with least-squares refined parameters of a = 16.13 (± 0.04) Å, b = 11.66 (± 0.02) Å, and c (fibre repeat) = 15.48 (± 0.02) Å. The presence of only a fourth-order meridional reflection indicated a four-fold helix. This fact, together with the

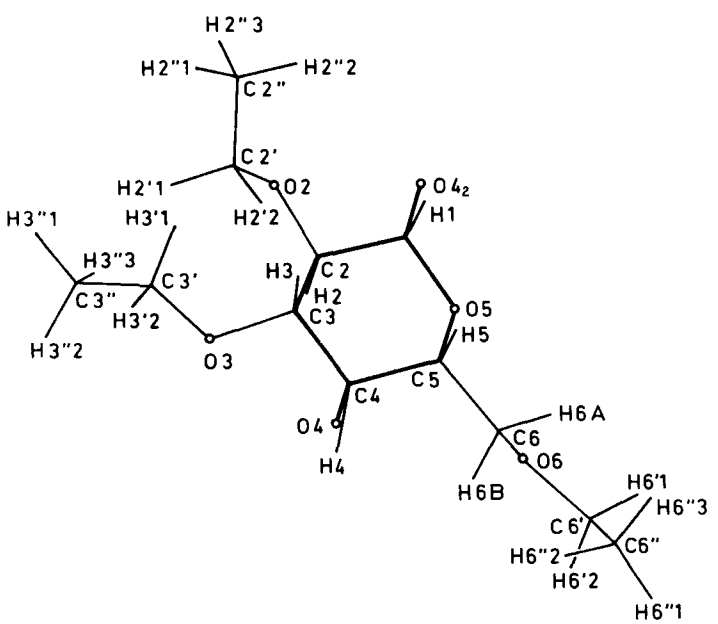


Fig. 3. Representation of one residue of tri-*O*-ethylamylose, showing atom labeling.

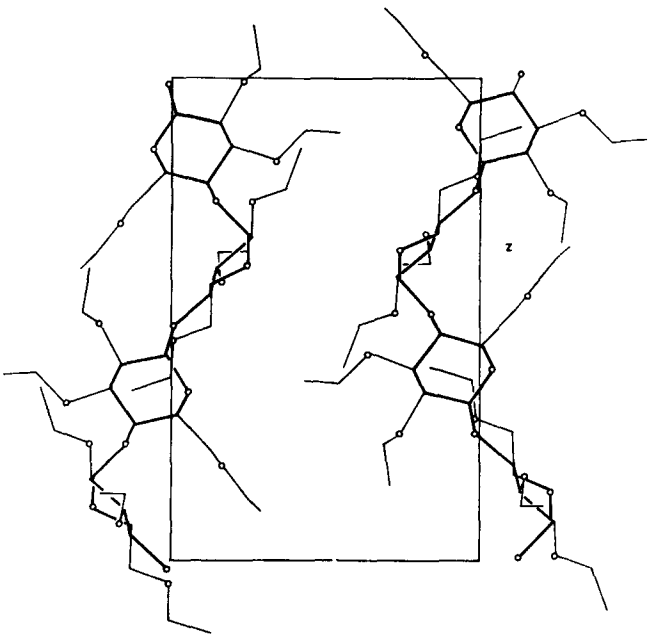


Fig. 4. View of two tri-*O*-ethylamylose chains on the $\bar{1}10$ plane.

measured density, indicated that two polymer chains must pass through the unit cell. From the electron micrographs of TEA single crystals, it was determined that the angle between two growth planes was 69°. This value is in agreement with the angle between planes 110 and $\bar{1}10$ in the proposed unit-cell. Debye–Scherrer patterns obtained from the single crystals were in agreement with data obtained from fibre diagrams of TEA.

RESULTS AND DISCUSSIONS

The method of stereochemical model analysis used in this investigation has been previously described in detail[2,4,6]. The standard valence bonds and angles for the α-D-glucopyranose ring were those of Arnott and Scott[7]. Pendant atoms were attached to the ring to complete the residue structure. The ring was in the $^4C_1$ conformation. The conformation of the TEA chain was refined, maintaining the fibre repeat and the four-fold helical symmetry. It was found that two rotational positions about the virtual bond* (O-4–O-4_2) resulted in a reasonable glycosidic bond angle of 114–123°. However, one of the positions was rejected because of unreasonably short, intramolecular, non-bonded contact distances. Also, in conformational analysis, only the *tg* and *gt*** positions of the EtO-6 group were sterically allowed,

*For a description of virtual-bond terminology, see Ref. 3.

**For example, *tg* means trans to O-5 and gauche to C-4, and similarly for *gt*.

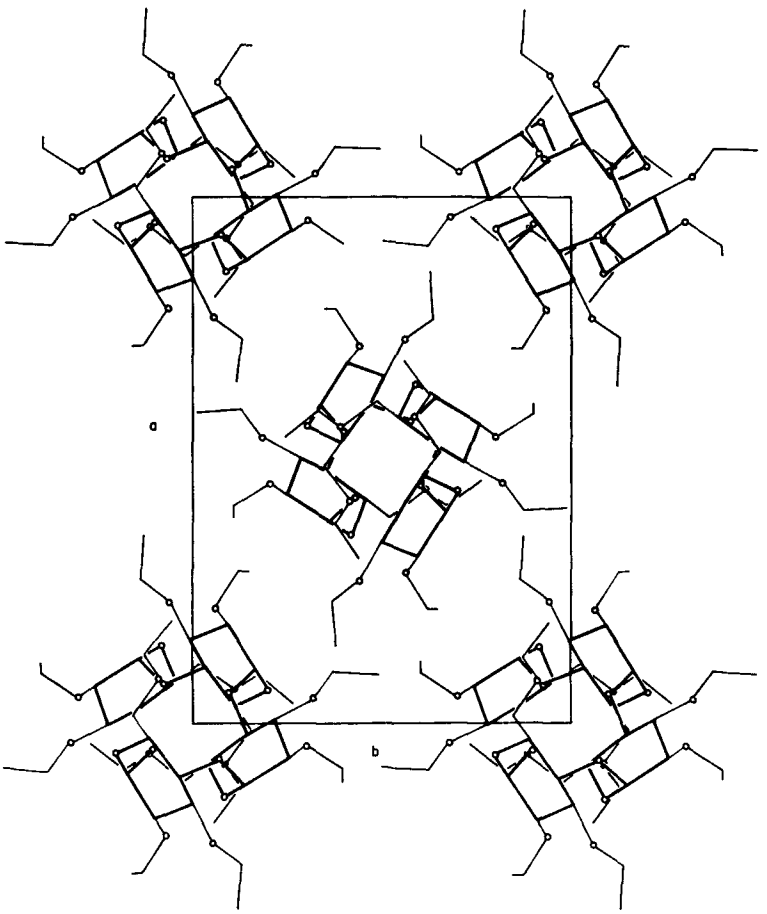


Fig. 5. View of *a–b* plane of tri-*O*-ethylamylose.

as previously observed in TMA[4]. Likewise, helices of right-handed chirality were found to be disallowed due to the presence of short intramolecular contacts.

In subsequent packing analysis, the O-6 *gt* position was found to be disallowed because of short intermolecular contacts. The packing analysis was performed in space-group $P2_12_12_1$, with portions of two chains in the unit cell. Since only a fourth-order meridional reflection was observed, the four-fold helix symmetry was maintained in the packing analysis.

Refinement of the resulting best model from packing and conformational analysis against X-ray structure amplitudes was then performed, using a method identical to that previously described[2,4]. The refinement produced only small changes in the model. The final co-ordinates are reported in Table I. The initial position of the residue is defined with the vector pointing from the origin to O-4 along the $-y$ axis. Rotation of this vector about the z axis of the co-ordinate system and translation along the z axis produce the necessary helix rotation and translation. The co-ordinates

TABLE I

CARTESIAN COORDINATES OF FIRST RESIDUE[a] IN ANGSTROMS (VIRTUAL-BOND LENGTH ≙ 4.35 Å)

Atom	x	y	z	*Atom*	x	y	z
O-4	4.808	−1.169	−0.110	H-3	4.739	−1.960	2.280
C-1	2.068	−1.590	2.909	H-4	3.074	−2.296	−0.161
C-2	2.850	−2.857	2.570	H-5	3.354	0.204	1.437
C-3	4.048	−2.509	1.711	H-6A	1.242	0.674	0.006
C-4	3.618	−1.688	0.500	H-6B	1.968	−0.403	−1.046
C-5	2.761	−0.485	0.911	H-2′1	3.103	−5.457	3.945
C-6	2.173	0.270	−0.266	H-2′2	1.694	−4.679	3.588
O-2	3.290	−3.445	3.790	H-3′1	5.292	−3.945	3.177
O-3	4.694	−3.700	1.253	H-3′2	6.545	−4.030	2.019
$O\text{-}4_2$	2.861	−0.778	3.760	H-6′1	1.559	2.410	−1.615
O-5	1.669	−0.914	1.744	H-6′2	2.357	1.398	−2.658
O-6	3.026	1.316	−0.748	H-2″1	1.834	−5.592	5.896
C-2′	2.556	−4.593	4.183	H-2″2	1.396	−3.936	5.819
C-2″	2.166	−4.628	5.645	H-2″3	2.998	−4.379	6.236
C-3′	5.549	−4.295	2.221	H-3″1	5.898	−6.216	3.007
C-3″	5.409	−5.794	2.179	H-3″2	5.838	−6.161	1.294
C-6′	2.516	2.048	−1.848	H-3″3	4.392	−6.052	2.203
C-6″	3.408	3.210	−2.293	H-6″1	3.108	3.535	−3.246
H-1	1.220	−1.842	3.475	H-6″2	4.408	2.891	−2.330
H-2	2.228	−3.524	2.050	H-6″3	3.320	4.002	−1.609

[a]The residue has been shifted 1/4 *a* as is necessary for space group $P2_12_12_1$, rotated 33.6°, and shifted −0.11 Å along the *z* axis as compared with the standard position of O-4 in Ref. 2 as (o, −y, o).

presented in Table I have been transformed to the final helix rotational and translational position. Bond lengths, bond angles, and torsion angles are listed in Table II. The resulting glycosidic bond angle was 121.9°, and the optimal virtual-bond length was 4.35 Å. The rotational position of EtO-6 was −32° removed from the exact *tg*-position. The final *R* factor, including unobserved reflections, was 0.33 and was calculated as follows:

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

where $|F_o|$ denotes the observed structure amplitudes, and $|F_c|$ the calculated structure amplitudes. The refined anisotropic temperature factors did not vary significantly from the isotropic value of 5, *i.e.*, $B_x = B_y = B_z = 5$; temperature factor = $\exp(-B \cdot \sin^2\theta)$. The calculated and observed structure amplitudes are reported in Table III, and the shortest inter- and intra-molecular contacts in Table IV.

CONCLUSION

It is of interest to compare the results of this investigation with those for other α-D-glucans and their derivatives. In V-amylose, the rise per residue is 1.32 Å, differing from those of 3.75 Å in ATA, 3.91 Å in TMA, and 3.87 Å found in this

TABLE II

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

Bond lengths (Å)		*Torsion angles (degrees)*	
O-4–C-4	1.434 (0.008)	O-5–C-1–C-2–C-3	56.9 (0.9)
C-4–C-3	1.525 (0.002)	C-1–C-2–C-3–C-4	−52.7 (0.5)
C-4–C-5	1.534 (0.009)	C-2–C-3–C-4–C-5	51.5 (−1.5)
C-1–C-2	1.526 (0.003)	C-3–C-4–C-5–O-5	−52.4 (3.0)
C-1–O-4$_2$	1.405 (−0.009)	C-4–C-5–O-5–C-1	57.9 (−3.2)
C-3–C-2	1.418 (0.003)	C-5–O-5–C-1–C-2	−60.7 (1.5)
C-5–O-5	1.515 (−0.006)	O-4–C-4–C-5–O-5	−169.5
C-2–O-2	1.439 (0.003)	O-4–C-4–C-3–C-2	168.6
C-3–O-3	1.424 (0.001)	O-4$_2$–C-1–C-2–C-3	−67.3
C-5–C-6	1.430 (0.001)	O-4$_2$–C-1–O-5–C-5	61.5
C-6–O-6	1.517 (0.003)	O-5–C-5–C-6–O-6	148.2
	1.434 (0.007)	C-4$_2$–O-4$_2$–C-1–H-1	−55.7
		C-1–O-4$_2$–C-4$_2$–H-4$_2$	−40.3

Bond angles (degrees)	
O-4–C-4–C-3	107.4 (1.9)[b]
O-4–C-4–C-5	107.2 (−1.4)
C-3–C-4–C-5	111.6 (1.3)
C-4–C-3–C-2	110.5 (0.0)
C-3–C-2–C-1	109.9 (−0.6)
C-4–C-5–O-5	110.2 (0.2)
C-5–O-5–C-1	114.0 (0.0)
C-2–C-1–O-5	111.2 (2.0)
C-2–C-1–O-4$_2$	108.8 (0.4)
O-5–C-1–O-4$_2$	112.4 (0.8)
C-3–C-2–O-2	109.6 (−1.2)
C-1–C-2–O-2	108.1 (−1.2)
C-4–C-3–O-3	108.8 (−0.9)
C-2–C-3–O-3	110.3 (0.7)
C-4–C-5–C-6	113.5 (0.8)
O-5–C-5–C-6	107.6 (0.7)
C-5–C-6–O-6	113.1 (1.3)
C-1–O-4$_2$–C-4$_2$	121.9

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′	104.5
C-2′–C-2″	1.514	C-2–O-2–C-2′–C-2″	−135.0
O-3–C-3′	1.422	O-2–C-2′–C-2″–H-2″1	−166.8
C-3′–C-3″	1.506	C-2–C-3–O-3–C-3′	158.4
O-6–C-6′	1.417	C-3–O-3–C-3′–C-3″	139.9
C-6′–C-6″	1.531	O-3–C-3′–C-3″–H-3″1	−169.4
		C-5–C-6–O-6–C-6′	180.0
		C-6–O-6–C-6′–C-6″	176.7
		O-6–C-6′–C-6″–H-6″1	165.0

Bond angles (degrees)	
C-2–O-2–C-2′	114.3
O-2–C-2′–C-2″	114.8
C-3–O-3–C-3′	113.7
O-3–C-3′–C-3″	110.0
C-6–O-6–C-6′	115.1
O-6–C-6′–C-6″	114.1

[a]Deviations from Arnott and Scott[7] average values are shown in brackets. [b]Derivation from average value for α-D-glucans; see Ref. 2. [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*	$\|F_{obs}\|$	$\|F_{calc}\|$
110	337	209	104[a]	7	10
200	140	134	014[a]	12	8
210[a]	16	3	114, 204	158	129
020	25	45	214, 024		
120[a]	23	0	124, 304	120	152
310	35	38	314, 224	148	93
220	139	130	404, 324		
400, 320	61	47	034, 414		
230[a]	37	57	134	88	184
			234[a]	43	59
101	124	109	424[a]	45	35
011[b]	109	111	504[a]	47	70
111	160	184	334[a]	48	11
201	42	24	514[a]	48	66
211	70	105	144, 044		
021	86	75	524, 434	66	110
121[b]	53	102			
301[a]	35	61	105[c]	15	9
311	235	215	015[c]	15	22
221	342	166	115, 205	30	22
401[a]	32	4	215[a]	22	34
321[a]	29	15	025, 125		
031, 411			305	84	73
131	102	87	315, 225	96	83
231[a]	35	68	405, 325	35	78
421	49	66	035, 415		
501, 331			135	51	79
511	105	90	235[a]	46	20
041, 141	78	54	425, 505	77	60
521[a]	47	38	335, 515	128	94
431[a]	48	34			
241, 601	67	69	106[c]	15	6
			016[c]	15	3
102	36	48	116[c]	15	42
012[b]	35	58	206[c]	15	35
112	53	56	216[a]	20	24
202	77	102	026[a]	25	28
212, 022			126, 306		
122	193	152	316, 226	48	82
302[a]	24	0	406, 326		
312, 222	321	394	036, 416		
402, 322	45	43	136	73	79
032, 412			236[a]	50	36
132	82	111	426, 506		
232[a]	38	91	336	118	152
422, 502					
332, 512	77	83			
103[a]	8	21			
013[a]	23	46			

TABLE III (continued)

113[a]	28	56
203, 213		
023	150	123
123[a]	25	8
303[a]	25	64
313, 223	197	178
403, 323	74	48
033, 413		
133	141	106
233[a]	40	47
423, 503	53	99
333, 513	96	44

[a]Unobserved structure amplitudes. [b]Unobserved structure amplitudes occurring in regions where layer line-streaking exists. [c]Unobserved structure amplitudes occurring in regions where the Lorenz factor is not well known for fibre diagrams.

TABLE IV

SHORTEST INTERMOLECULAR AND INTRAMOLECULAR CONTACTS*

Intermolecular contact	*Distance (Å)*[b]	
C-2′ ... C-3″	3.70	
H-2′3 ... O-3	2.89	all contacts involve
H-3″3 ... C-3″	2.92	the symmetry element
C-2′ ... H-2	3.01	$\bar{x}$, 1/2 + y, 1/2 − z
H-3″3 ... C-2′	3.03	
H-3″3 ... H-3″3	2.43	
H-2′3 ... H-3″3	2.47	

Intramolecular contact	*Distance (Å)*[b]
O-2 ... $O\text{-}4_2$[a]	2.70
C-3 ... $O\text{-}4_2$[a]	2.93
C-5 ... $O\text{-}4_2$[a]	2.87
O-5 ... $C\text{-}4_2$[a]	3.02
C-1 ... $O\text{-}3_2$	2.96
H-1 ... $O\text{-}3_2$	2.25
C-1 ... $C\text{-}3_2$[a]	3.15
H-1 ... $C\text{-}4_2$[a]	2.72
C-1 ... $H\text{-}4_2$[a]	2.66
H-5 ... $H\text{-}6B_2$	2.29

[a]1 ... 4 contacts. [b]All distances not due to 1 ... 4 contacts are greater then the minimum contact distances as set forth in Ref. 8.
*See also note added in proof, p. 250.

investigation for TEA. The conformation of V-amylose is approximated by a six-fold left-handed helix (in helix notation, a 6_5 helix), while those of the derivatives are a 14_{11} helix for ATA, approximated by a 4_3 helix for TMA, and a 4_3 helix for TEA. It has been proposed that the difference in helix symmetries is due to the existence of an O-2....$O\text{-}3_2$ hydrogen-bond in V-amylose, whereas this interaction

cannot result in a hydrogen bond in the structures of the derivatives[4]. As expected, the conformations of TMA and TEA are very similar. In TMA, a two-fold screw axis exists in the chain direction, as the MeO-6 group is in different rotational positions on adjacent residues. In V-amylose, a two-fold screw axis is found along the chain for similar reasons. However, in TEA, conformation and packing criteria, as well as the X-ray data, accord well with a four-fold helix in the orthorhombic unit-cell. Very few intermolecular short-contacts in the interstitial spaces between adjacent helices are found. This might be one reason for the ability of TEA to incorporate guest molecules easily in the crystalline lattice.

ACKNOWLEDGMENTS

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Note added in proof (November 11th, 1977). — Table IV does not contain contacts from the first to the third residue. However, the best model obtained by X-ray refinement resulted in some very short contacts between O-2 of the first residue and the MeC-6″ group of the third residue. These short contacts can be released by altering the torsion angle defining O-6 from 148.2 to 145° (*cf.* Table II) and those of the linked ethyl group, namely the angles defining C-6′ to −173°, C-6″ to 150°, H-6″1 to 136°, and consequently H-2″1 to −106° and H-3″1 to 176°. A helix rotation of 1.6° and helix shift in z of 0.17 Å of the co-ordinates of Table I resulted then in the best R value of 0.29 for the observed reflections, and 0.36 for all reflections. The only short contacts remaining between the first and the third residue are O-2 ... C-6″$_3$ (2.99 Å), and H-3′2 ... H-6″1$_3$ (1.97 Å).