

On a Possible Substitution of *p*-Xylene by Toluene in *p*-Xylene Crystals. The Crystal Structure of *p*-Xylene, C₈H₁₀, at 180 K

BY H. VAN KONINGSVELD AND A. J. VAN DEN BERG

Laboratory of Applied Physics, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands

J. C. JANSEN

Laboratory of Organic Chemistry, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands

AND R. DE GOEDE

Laboratory of Process Equipment, Delft University of Technology, Leeghwaterstraat 44, 2628 CA Delft, The Netherlands

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Abstract

$M_r = 106.17$, monoclinic, $P2_1/n$, $a = 5.806(2)$, $b = 5.023(1)$, $c = 11.215(2)$ Å, $\beta = 100.48(2)^\circ$, $V = 321.61(14)$ Å³, $Z = 2$, $D_x = 1.096$ g cm⁻³, $\lambda(\text{Mo } K\alpha) = 0.71069$ Å, $\mu = 0.66$ cm⁻¹, $F(000) = 116$, $T = 180$ K, $R = 0.045$ for 926 reflections [$I > 2.5\sigma(I)$]. The cell dimensions are confirmed by powder diffraction. No phase transition is observed between 110 and 273 K. The crystal data reported by Biswas [*Indian J. Phys.* (1960), **34**, 263–271] are incorrect. The bond lengths and bond angles in *p*-xylene are equal to the corresponding values in toluene within experimental error. The aromatic ring is essentially planar and slightly deformed at the substituent side. The centre of symmetry in *p*-xylene coincides with the space-group centre. The *p*-xylene molecules are packed into chains along **b** by nearly equal van der Waals contacts between the six ring C atoms and one methyl H of a neighbouring molecule. The chains are held together by van der Waals forces. Because of the great resemblance of packing in *p*-xylene and α -toluene crystals, the possibility of the existence of solid solutions of toluene in *p*-xylene is proposed. This proposal is verified by measuring the *p*-xylene-rich part of the phase diagram of the toluene/*p*-xylene system. Separation of pure *p*-xylene crystals from a mixture containing toluene seems unlikely.

Introduction

p-Xylene is an important intermediate for the production of polyethyleneterephthalate. On an industrial scale, separation of *p*-xylene from a mixture of (among others) its aromatic isomers by crystallization is widely applied. Crystallization is carried out in two stages at 228 and 213 K. Each stage consists of an

adiabatic well mixed crystallizer. The removal of heat takes place in scraped surface heat exchangers in a recycle loop. The crystals are separated from the liquid phase by centrifuges. The problem in practice is that the crystals are rather small. This implies that the amount of mother liquid attached to the crystal's surface is rather high. It is therefore important to increase the crystal size thereby increasing the production capacity of the plant. Mixed-crystal formation, however, would have dramatic consequences for the purity of the product: during the washing operation recrystallization would occur, the rate and effectiveness of which would decrease with increasing crystal size. For the theoretical part of the project and the modelling of the plant it is important to know growth phenomena of *p*-xylene crystals. A theoretical treatment (*i.e.* periodic-bond-chains analysis) requires knowledge of the crystal structure. The X-ray structure determination is described in this paper. Powder diffraction is used to verify whether or not the *p*-xylene packing changes with temperature. Finally, preliminary measurements of the *p*-xylene-rich part of the phase diagram of *p*-xylene/toluene are performed. Mixed crystals can be expected because of the great resemblance of the packing in *p*-xylene and α -toluene crystals. Gas-chromatographic analysis is used because mixed crystals, containing small amounts of toluene, cannot be studied adequately by X-ray diffraction.

Experimental

Single-crystal structure determination

Crystals were grown under different conditions. First, a sample of the feed-stream was taken at the plant of Esso Nederland BV, The Netherlands. From

this mixture crystals were grown [crystal (I)] at ≈ 235 K. A second crystal [crystal (II)] was grown at ≈ 268 K from a mixture of 70 vol.% *p*-xylene and 30 vol.% *m*-xylene. Finally, crystals were grown from pure *p*-xylene at 286 K [crystal (III)] and by sublimation at 273 K [crystal (IV)]. Preliminary measurements with all crystals at 260 K and at 110 K showed essentially the same (contracted) cell dimensions. Results for crystal (IV) are given in the *Abstract*.

Crystal (IV), approximate size $0.4 \times 0.3 \times 0.2$ mm, most prominent faces: {001}, was enclosed in a glass capillary and sealed with 1266 STYCAST glue. The measuring temperature of 180 K was obtained by blowing a temperature-controlled stream of nitrogen along the crystal. At lower temperatures ice deposits on the glass capillary seriously influence the accuracy of the measurements. Systematic extinctions, $h0l$ for $h+l = \text{odd}$ and $0k0$ for $k = \text{odd}$, indicated space group $P2_1/n$. The cell parameters were obtained from diffractometer angular settings of 25 centred reflections with $15.4^\circ < \theta < 22.8^\circ$. Data collected for $hk \pm l$ ($h_{\max} = 8$, $k_{\max} = 7$, $l_{\max} = 14$) in $\omega/2\theta$ scan mode up to $\theta = 32.0^\circ$ on a Nonius CAD-4 diffractometer using graphite-monochromated $\text{Mo K}\alpha$ radiation. Scan width = $(0.85 + 0.35 \tan \theta)^\circ$. Maximum recording time 120 s, $\sigma_{\text{count}}(I)/I < 0.02$ requested in a scan. An effect of the solubility of STYCAST in *p*-xylene was observed by a change in the peak shape and by the decay of 9% in the intensity of four standard reflections which were monitored after each 3600 s of X-ray measuring time. The data were corrected accordingly. These gave 1114 independent data of which 926 reflections with $I > 2.5\sigma(I)$ were used in subsequent calculations. Extinction and absorption were neglected. The structure was solved by the Patterson method because direct methods failed; H atoms located from a difference map. Least-squares refinement (anisotropic C atoms, isotropic H atoms) on F converged to $R = 4.5\%$, $wR = 5.0\%$, $w = 1$, for 926 observed reflections, $S = 1.51$. $(\Delta/\sigma)_{\max} = 0.03$ [y value of H(2)]. The final difference map showed four peaks of about $0.27 \text{ e } \text{\AA}^{-3}$ half-way between bonded C atoms. All other peaks were lower than $0.13 \text{ e } \text{\AA}^{-3}$. Atomic scattering factors from XRAY72 (Stewart, Kruger, Ammon, Dickinson & Hall, 1972). The calculations were performed on the Delft University Amdahl 470/V7B computer using XRAY72 (Stewart *et al.*, 1972).

Powder diffraction analysis

Crude crystals of pure *p*-xylene, grown by sublimation, were crushed and mixed with corundum as internal standard in a refrigerating chamber at 268 K. This mixture was plugged in a Lindemann-glass capillary of 0.2 mm \varnothing which was mounted on a special sample holder with a Peltier cooling device (Fraase Storm & Tuinstra, 1986) for the Guinier-Simon

camera (Enraf-Nonius). At a number of temperatures between 243 and 283 K diagrams were recorded using $\text{Cu K}\alpha_1$ radiation ($\lambda = 1.54051 \text{ \AA}$), exposure time 8 or 10 h. Lower temperatures (down to 133 K) were obtained by cooling the sample in a cold stream of nitrogen. From the diagrams at 243 and 273 K intensities and positions of the diffraction lines were determined with an optical microdensitometer. Cell constants were refined with a least-squares procedure using all measured reflections.*

Preliminary phase-diagram measurements

The *p*-xylene-rich part of the phase diagram of the *p*-xylene/toluene system was measured using the equipment presented schematically in Fig. 1.

Mixtures of *p*-xylene and toluene (≈ 500 ml) of different composition were stirred successively in a 1 l vessel. The temperature was lowered (coolant methanol) until nucleation occurred. Next, the temperature was raised just until a small amount of crystals was left. Then, the temperature was lowered slowly allowing the crystals to grow with as few occlusions of liquid as possible. The mixture was held at constant temperature for about 4 h. After equilibration the slurry was fed into a pre-cooled centrifuge. The temperature difference between the stirred vessel and the pre-cooled centrifuge was kept within 0.5 K. Filter paper was placed in the centrifuge drum to prevent the escape of small crystals. After separation of the liquid from the crystals, the crystal cake was washed with cold ethanol to remove the mother liquid as well as possible. The ethanol was kept at the same temperature as the equilibrated mixture. Samples

* Lists of powder diffraction data, the GC analysis, Table 4, structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 42805 (8 pp.) Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

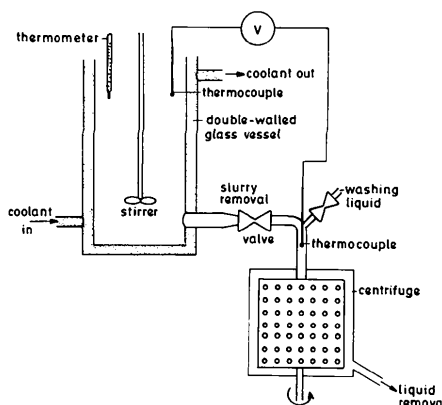


Fig. 1. Schematic drawing of the equipment used for the measurement of solid-liquid equilibria.

Table 1. *Positional and thermal parameters* ($\text{\AA}^2 \times 10^4$) *with e.s.d.'s in parentheses*

$$U_{\text{eq}} = \frac{1}{3}(U_{11} + U_{22} + U_{33})$$
. Isotropic U value for H atoms.

	x	y	z	U_{eq}/U
C(1)	0.6063 (2)	0.3087 (2)	0.4348 (1)	225 (3)
C(2)	0.3833 (2)	0.4077 (3)	0.3893 (1)	253 (4)
C(3)	0.2792 (2)	0.5952 (3)	0.4536 (1)	258 (4)
C(4)	0.7174 (3)	0.1006 (3)	0.3673 (1)	304 (5)
H(2)	0.296 (3)	0.340 (4)	0.308 (2)	356 (46)
H(3)	0.124 (3)	0.661 (4)	0.420 (1)	355 (46)
H(41)	0.659 (4)	0.103 (5)	0.276 (2)	676 (68)
H(42)	0.889 (4)	0.126 (5)	0.372 (2)	675 (68)
H(43)	0.696 (3)	-0.076 (4)	0.393 (2)	551 (61)

from the liquid and solid phase were analysed by GC.*

Results

Molecular structure and crystal packing

The final atomic positions and thermal parameters of *p*-xylene are given in Table 1.* The molecular structure and atomic numbering are given in Fig. 2. Bond lengths, bond angles and torsion angles are listed in Table 2 together with the corresponding values in the 'averaged' toluene molecule (Anderson, Bosio, Bruneaux-Pouille & Fourme, 1977). The aromatic ring is essentially planar and slightly deformed at the substituent side. *p*-Xylene and toluene are geometrically very much alike. Even the rotational orientation of the methyl groups is the same in both molecules: one methyl H atom, H(43), deviates 7° from being perpendicular to the aromatic ring plane.

The packing of *p*-xylene in (001) is given in Fig. 3(a). H(43) has six nearly equal van der Waals contacts (distances between 3.06 and 3.18 Å) with the C atoms of the ring in a neighbouring molecule. The molecules thus form infinite chains along **b**. Five independent H...H contacts smaller than 3.02 Å link these chains together, so that layers are formed parallel to (001). The van der Waals contacts are summarized in Fig. 3(a) and Table 3(a).

* See deposition footnote.

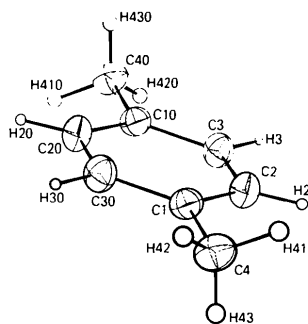


Fig. 2. ORTEP drawing (Johnson, 1965) of *p*-xylene. C(10) and C(1), etc., are centrosymmetrically related.

Table 2. *Molecular geometry of p-xylene at 180 K and the average geometry of α-toluene at 165 K*

Average geometry: arithmetic mean values of the two independent molecules assuming m symmetry within the molecules. Distances between heavy atoms corrected for libration at 165 K.

	<i>p</i> -Xylene	Toluene
Bond lengths (Å)		
C(1)-C(4)	1.503 (2)	1.505 (1)
C(1)-C(2)	1.394 (2)	1.398 (1)
C(2)-C(3)	1.389 (2)	1.389 (1)
C(3)-C(10)	1.393 (2)	1.385 (1)
C(2)-H(2)	1.02 (2)	0.99 (1)
C(3)-H(3)	0.97 (2)	0.97 (1)
C(4)-H(41)	1.01 (2)	0.98 (1)
C(4)-H(42)	0.99 (2)	0.95 (1)
C(4)-H(43)	0.95 (2)	0.98 (1)
Bond angles (°)		
C(2)-C(1)-C(4)	121.2 (1)	121.12 (5)
C(30)-C(1)-C(4)	121.0 (1)	121.12 (5)
C(30)-C(1)-C(2)	117.7 (1)	117.79 (5)
C(1)-C(2)-C(3)	121.0 (1)	121.05 (5)
C(2)-C(3)-C(10)	121.3 (1)	120.32 (5)
C(3)-C(10)-C(20)	117.7 (1)	119.47 (5)
C(1)-C(2)-H(2)	119 (1)	117 (1)
C(3)-C(2)-H(2)	120 (1)	122 (1)
C(2)-C(3)-H(3)	120 (1)	121 (1)
C(10)-C(3)-H(3)	119 (1)	119 (1)
C(1)-C(4)-H(41)	114 (1)	107 (1)
C(1)-C(4)-H(42)	113 (1)	107 (1)
C(1)-C(4)-H(43)	114 (1)	113 (1)
H(41)-C(4)-H(42)	102 (2)	114 (1)
H(41)-C(4)-H(43)	106 (2)	107 (1)
H(42)-C(4)-H(43)	107 (2)	107 (1)
Torsion angles		
C(2)-C(1)-C(4)-H(41)	27 (1)	21 (1)
C(30)-C(1)-C(4)-H(42)	39 (1)	36 (1)
C(30)-C(1)-C(4)-H(43)	83 (1)	83 (1)

The layers parallel to (001), related by a screw diad parallel to **b**, are held together by seven independent H...H contacts smaller than 3.02 Å. Table 3(b) summarizes the numbered interlayer contacts indicated in Fig. 4(a).

Powder diffraction

The combined results from powder diffraction analysis and single-crystal measurements are given in Table 4* and plotted in Fig. 5.

The absence of anomalies in the smooth curves clearly shows that there is no evidence for a phase transition in the region 110 to 273 K. Additional powder diagrams were taken in the region from 133 K to the melting point at 286 K. The presence of ice-lines in the lower-temperature region and of texture (caused by recrystallization) above 273 K seriously affected the accuracy of the cell dimensions obtained from these diagrams. These results are therefore not included in Table 4 and Fig. 5. The present cell, however, still allows indexing of these rather poor diagrams.†

* See deposition footnote.

† Available on request.

The cell dimensions do not agree with those reported by Biswas (1960), who used Debye-Scherrer powder diffraction data. We have repeated his experiment by cooling liquid *p*-xylene to 90 K. The Guinier-Simon diagram, very poor because of texture and

ice-lines, could hardly be indexed with our unit cell and did not resemble the published diagram of Biswas. In our opinion the Debye-Scherrer technique used by Biswas together with his method of sample preparation have led to a diagram which was inevitably interpreted incorrectly.

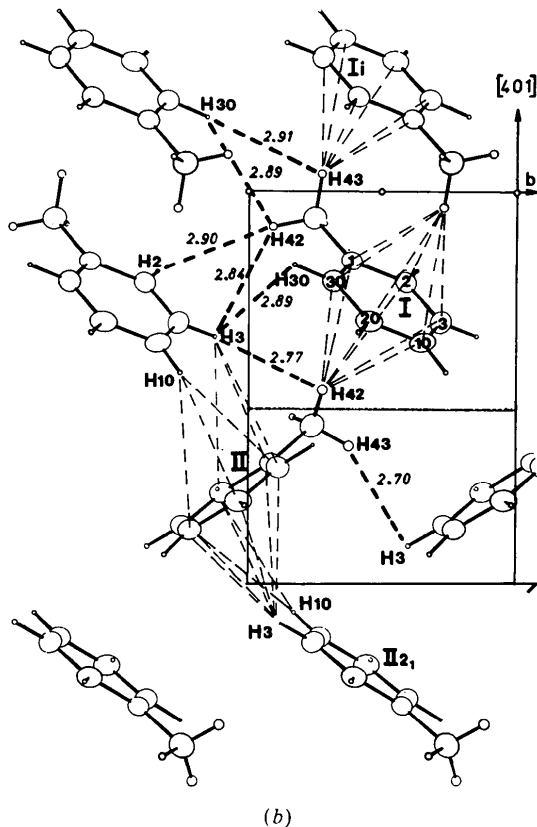
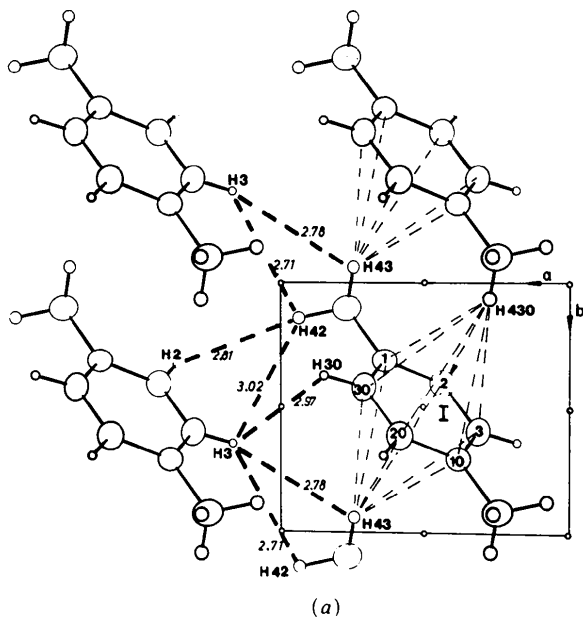


Fig. 3. Packing in layers. (a) *p*-Xylene: (001) layer. (b) α -Toluene: (104) layer.

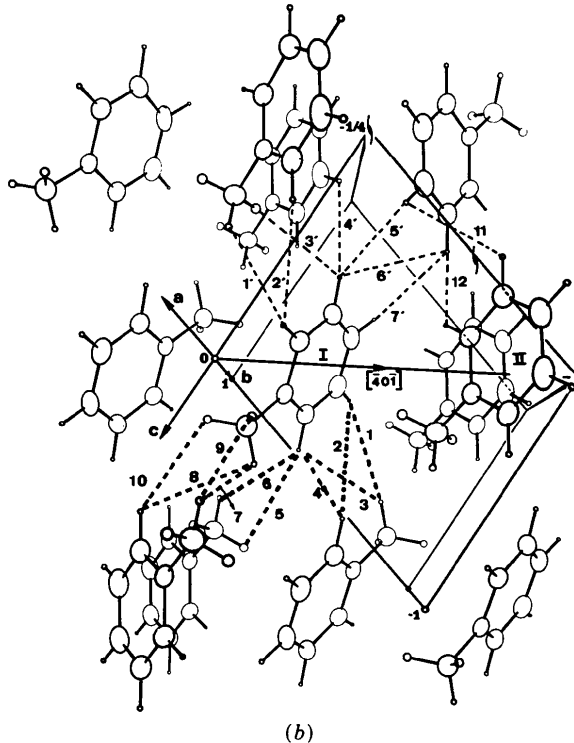
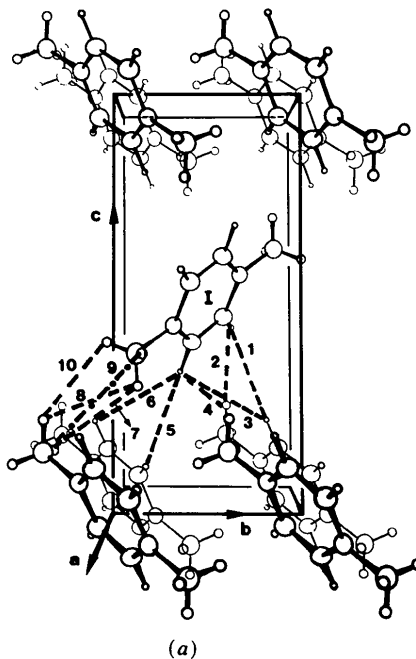


Fig. 4. Interlayer contacts. (a) *p*-Xylene. (b) α -Toluene.

Table 3. *van der Waals contacts in p-xylene and α -toluene crystals*E.s.d.'s for C...H \approx 0.02 Å and for H...H \approx 0.03 Å.(a) C...H contacts (< 3.6 Å) to ring C atoms in layers parallel to (001) and ($\bar{1}04$) in *p*-xylene and α -toluene respectively

Crystal	H atom*	C atoms in molecule		C(1)	C(2)	C(3)	C(10)	C(20)	C(30)
		I or II							
<i>p</i> -Xylene	H(I, 43, 1)	I		3.18	3.16	3.11	3.07	3.06	3.12
α -Toluene	H(I, 43, 2)	I		3.08	3.13	3.12	3.06	2.99	3.01
	H(II, 42)	I		3.05	3.05	3.17	3.27	3.25	3.14
	Average†			3.18	3.19	3.13	3.06	3.02	3.09
α -Toluene	H(II, 3, 3)	II		3.58	3.40	3.05	2.90	3.08	3.42
	H(II, 10, 3)‡	II			3.24	3.58			
	H(I, 3, 4)	II		3.08	3.59				3.36
	H(I, 10, 4)‡	II					3.55	3.09	3.17

(b) Interlayer H...H contacts smaller than 3.02 Å; for comparison some distances larger than 3.02 Å have been added; the numbers are referred to in Fig. 4

No.	1,1'	2,2'	3,3'	4,4'	5,5'	6,6'	7,7'	8	9	10	11	12
<i>p</i> -Xylene	2.84	2.52	2.84	2.95	2.84§	2.84§	2.90	2.83	>3.6	2.74		
α -Toluene	3.01	>3.6	2.62	2.68	3.00	2.75	3.00	3.08	2.71	2.86		
	2.75	3.19	2.74	2.96	2.62	2.87	2.82				2.90¶	2.49¶

* H(*n*, *m*, *i*) = H atom number *m* in molecule *n*, after symmetry operation *i*. The H atom is in the molecule at *x*, *y*, *z* when no value for *i* is given. Symmetry codes are: *i* = 1: *x*, *y* + 1, *z*; *i* = 2: $-x$, $-y + 1$, $-z$; *i* = 3: $-2 - x$, $y + \frac{1}{2}$, $-\frac{1}{2} - z$; *i* = 4: *x*, *y* - 1, *z*.

† Average: 3.08 and 3.27 (3.13 and 3.25, etc.) gives average value 3.18 (3.19, etc.); see text.

‡ No comparable proton in *p*-xylene bonded to C(10).§ Distances 5 and 6 in *p*-xylene are not independent but symmetry-related (via a 2_1 axis) to 1 and 3, respectively.

¶ Between two molecules II.

The cell dimensions listed in Table 4 were obtained with samples prepared by the rather unconventional method of powdering, in a refrigerating chamber, single crystals grown by sublimation. However, single crystals grown under different conditions (see *Experimental*) showed essentially the same cell dimensions.

Therefore, the crystal structure as described in this paper can be considered as the basic structure of the crystals grown in the Esso plant, where *p*-xylene is produced by the process described in the *Introduction*.

Phase diagram

The result of the experimental determination of part of the phase diagram of the *p*-xylene/toluene systems is presented in Fig. 6.

Morphology studies (de Goede, 1986) have shown that *p*-xylene crystals grown from a mixture of 70 mol% *p*-xylene and 30 mol% toluene still have the same basic structure as pure *p*-xylene. The present preliminary experiments were performed in mixtures containing up to 60 mol% toluene. We decided not to go beyond this, already extreme, limit because the feed stream from which *p*-xylene is separated at the Esso plant contains only a few per cent of toluene. Furthermore, it is questionable in case solid solutions are formed, whether the basic structure of *p*-xylene is preserved at higher toluene contents.

The accuracy of the measurements reported in Fig. 6 is rather uncertain: it is unpredictable how much liquid, despite the precautions taken, is occluded in the growing crystals. Moreover, the effectiveness of the washing procedure, repeated for each experiment in the same way, is unknown. Nevertheless, the measurements do not exclude the possibility of the formation of a solid solution. A complete description of the solid-phase behaviour, however, requires measurements over the whole composition range and a better estimate of the accuracy. It is intended to report the results of these measurements in the near future.

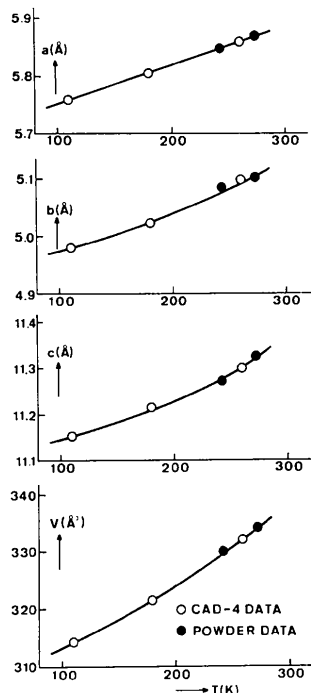


Fig. 5. Unit-cell constants and volume of *p*-xylene versus temperature. Combined results from powder data (●) and single-crystal measurements (○).

Discussion

Comparison of the packing of *p*-xylene and α -toluene* (Anderson *et al.*, 1977) revealed a striking resemblance. Fig. 3(b) shows the packing of α -toluene in $(\bar{1}04)$. The *b* axis in toluene is nearly equal to the *a* axis in *p*-xylene. The vector connecting the ring centres of two centrosymmetrically related molecules (molecules I and II) is almost parallel to $[401]$. The distance between the two ring centres is 4.93 Å, which value nearly equals the *b* axis in *p*-xylene (see Table 4). The orientation of molecules I and II around the center of symmetry is approximately identical to the orientation of molecules in *p*-xylene along $[010]$. As a consequence, between I and II in α -toluene, almost the same six C...H contacts (distances from 2.99 to 3.13 Å) exist as in *p*-xylene.† In *p*-xylene the C...H contacts are repeated at the other side of the ring by inversion. In α -toluene a second independent molecule (molecule II) orients itself in such a way as to produce six new C...H contacts with molecule I between 3.05 and 3.27 Å.‡ The methyl H atom involved is not the 'nearly perpendicular' one but the one rotated 36° (Table 2). If the C...H contacts at both sides of ring I are averaged assuming a local inversion centre in ring I, the resulting values are in perfect agreement with the corresponding values in *p*-xylene (Table 3a). The packing of molecules II in $(\bar{1}04)$ is less comparable with the packing in *p*-xylene. The H atoms involved in short

C...H contacts are those bonded to ring C atoms.* Again there are six C...H contacts between molecules related by a screw diad (II and II₁), involving one ring H atom. However, the C...H distances cover a much wider range: from 2.90 to 3.58 Å. At the other side of ring II each of two H atoms from ring I has three C...H contacts between 3.08 and 3.59 Å (Fig. 3b, Table 3a). As in *p*-xylene, five H...H contacts smaller than 3.0 Å link the molecules I in layers parallel to $(\bar{1}04)$. A sixth H...H contact [between H(42) in molecule II and H(3) in a $-\mathbf{b}$ -translated molecule I] is equal to a H(43)...H(3) contact in *p*-xylene. One short H...H contact (2.70 Å) exists between two II molecules one *b* apart. This contact replaces an H(42)...H(3) contact of the same length in *p*-xylene (see Fig. 3b).

The layers parallel to $(\bar{1}04)$, related by a screw diad parallel to *b* (note: corresponding to the *a* direction in *p*-xylene), are held together by sixteen H...H contacts smaller than 3.02 Å. Fig. 4(b) gives the independent part of the three-dimensional packing in α -toluene seen down *b* together with the numbered interlayer contacts (see Table 3b). Although the orientation of the layers with respect to each other is completely different the packing in both crystals still looks very similar.

In conclusion one can say that in this kind of structure the (methyl) H atom plays an important role in what can be recognized as a fundamental interaction with the aromatic ring atoms. This fundamental interaction is present in $\{001\}$ plates in *p*-xylene as well as, locally, in $\{\bar{1}04\}$ plates in α -toluene. In addition, the H...H contacts in both crystals are very similar. Consequently, preferential adsorption of toluene on the most prominent faces of *p*-xylene might occur. Once bound, toluene influences the deposition of oncoming layers so changing the rates of growth in these directions (Wang, Berkovitch-Yellin & Leiserowitz, 1985). A significant influence of toluene on the growth rate and habit of *p*-xylene crystals has indeed been observed (de Goede, 1986). Separation of pure *p*-xylene by crystallization from a feed stream containing a few per cent toluene seems therefore unlikely. The preliminary measurements of the phase diagram of *p*-xylene/toluene in the region of low toluene content are not in contradiction with this statement.

* α -Toluene crystallizes in $P2_1/c$ with two independent molecules (molecules I and II) and $a = 7.666$, $b = 5.832$, $c = 26.980$ Å and $\beta = 105.73^\circ$, $T = 165$ K. No e.s.d.'s given. Accuracy estimated to be of the same order of magnitude as in the present *p*-xylene structure. Molecules I and II are obtained by applying the operations $-x$, $-y+1$, $-z$ and $x-1$, $\frac{1}{2}-y$, $z-\frac{1}{2}$, respectively, to the published coordinates.

† The contraction in C...H contacts owing to the difference in measuring temperature is well within the standard deviation in C...H distances.

‡ Not included in Table 4 of Anderson *et al.* (1977). Calculated from the published coordinates. Their Table 4 contains some errors: H(112) should replace H(113) and H(24) should replace H(212).

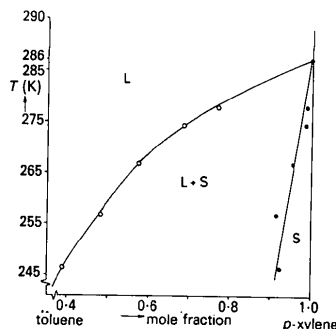


Fig. 6. Part of the solid-liquid equilibria in *p*-xylene/toluene.

* These contacts seem to play an important role in the packing of the metastable phase of toluene: β -toluene (Andre, Fourme, Bruneaux-Poulle & Bosio, 1982).

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The Refined Structure of Beef Liver Catalase at 2.5 Å Resolution

BY IGNACIO FITA,* ABELARDO M. SILVA,† MATHUR R. N. MURTHY‡ AND MICHAEL G. ROSSMANN

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, USA

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Abstract

The crystal structure of beef liver catalase [Murthy, Reid, Sicignano, Tanaka & Rossmann (1981). *J. Mol. Biol.* 152, 465-499] has now been refined by a restrained parameter least-squares method [Konnert & Hendrickson (1980). *Acta Cryst.* A36, 344-350] with respect to 2.5 Å data. Some extra density was discovered during the refinement process. This was interpreted in terms of a bound NADP molecule [Kirkman & Gaetani (1984). *Proc. Natl Acad. Sci. USA*, 81, 4343-4348; Fita & Rossmann (1985). *Proc. Natl Acad. Sci. USA*, 82, 1604-1608]. When the non-crystallographic symmetry was imposed as a constraint, the *R* factor was reduced to 21.2%. However, refinement of the two crystallographic independent subunits gave a final *R* factor of 19.1%. The refined coordinates have been re-analyzed for main-chain and side-chain hydrogen bonding, charge distribution, secondary structural element interactions, subunit contacts and molecular packing. The fractional accessibility and the temperature-factor variation are also discussed. The oligomerization process is considered in terms of the unusual quaternary structure. The organization of the heme channel and its relation to the enzyme's catalytic properties have been discussed elsewhere [Fita & Rossmann (1985). *J. Mol. Biol.* 185, 21-37].

Introduction

The biological function of catalase (EC 1.11.1.6) is still unclear, as is also the *in vivo* role of the bound

* Present address: Departamento de Química Macromolecular, ETSIIB, Diagonal 647, 08028 Barcelona-28, Spain.

† Present address: Departamento de Física, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CC No. 67, 1900 La Plata, Argentina.

‡ Present address: Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India.

NADP molecule found in some mammalian catalases (Kirkman & Gaetani, 1984; Fita & Rossmann, 1985*a*) and the extra 'flavodoxin-like' domain in fungal catalases (Vainshtein, Melik-Adamyán, Barynin, Vagin & Grebenko, 1981). However, the presence of catalase in most aerobic organisms has been related to the protection of cells from the toxic effects of small peroxides (*cf.* Deisseroth & Dounce, 1970; Schonbaum & Chance, 1976).

The structure of beef liver catalase (BLC) had been determined (Reid, Murthy, Sicignano, Tanaka, Musick & Rossmann, 1981; Murthy, Reid, Sicignano, Tanaka & Rossmann, 1981) and bears a close resemblance to fungal catalase (Vainshtein *et al.*, 1981). In this paper we describe the refinement of the atomic parameters and analyze the molecular structure. Elsewhere (Fita & Rossmann, 1985*b*) we have discussed the heme environment in relation to the catalytic properties of the enzyme.

Refinement of the structure

(a) Crystal and molecular data

The molecular weight of BLC is 235 000. There are four polypeptide chains per molecule, each with one heme group and one NADP moiety (Kirkman & Gaetani, 1984; Fita & Rossmann, 1985*a*). Each subunit has usually 506 amino acids, although some beef liver extracts have been reported with 10 to 15 additional residues (Schroeder, Shelton, Shelton, Robberston, Apell, Fang & Bonaventura, 1982; Schroeder, Shelton, Shelton, Apell, Evans, Bonaventura & Fang, 1982). The crystals are trigonal, have space group *P*3₂21 with *a* = 142.0 and *c* = 103.7 Å and were first reported by Eventoff, Tanaka & Rossmann (1976). The molecule is positioned on a crystallographic diad with two subunits per crystallographic asymmetric unit. For most of the refinement, the coordinates were