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Clarithromycin form I determined by synchrotron X-ray powder diffraction

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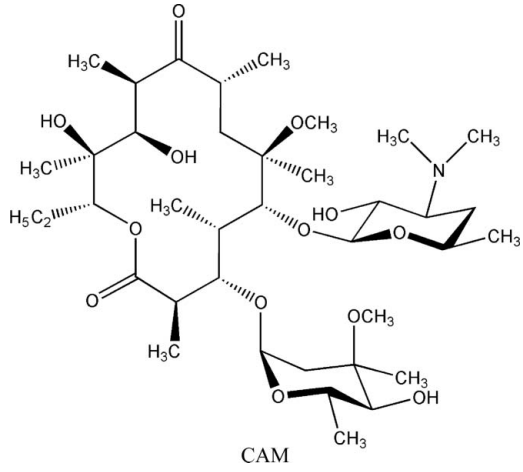
The structure of the metastable form I polymorph of the macrolide antibiotic clarithromycin, $C_{38}H_{69}NO_{13}$, was determined by a powder diffraction method using synchrotron radiation. The space group of form I is $P2_12_12$. The initial model was determined by a molecular replacement method using the structure of clarithromycin form 0 as a search model, and the final structure was obtained through Rietveld refinements. In the form I crystal structure, the clarithromycin molecules are aligned parallel along the *a* axis in a head-to-tail manner with intermolecular hydrogen bonds between the hydroxy O atoms. The dimethylamine groups of the clarithromycin molecule interdigitate between neighbouring head-to-tail clarithromycin alignments. The novel crystal packing found in form I provides a mechanism that describes the transformation of form 0 to form I.

Comment

Clarithromycin (CAM) is a 14-membered semisynthetic macrolide antibiotic widely used for the treatment of infections with various bacteria, including chlamydia, mycoplasma and *Helicobacter pylori*. CAM exists in eight different crystal forms, designated form 0 (ethanol solvate; Spanton *et al.*, 1999), form I (Liu *et al.*, 1999), form II (Liu & Riley, 1998), form III (acetonitrile solvate; Liu *et al.*, 2003), form IV (water solvate; Avrutov *et al.*, 2003), form V (Gruss, 2008), the hydrochloride salt (Parvez *et al.*, 2000) and the methanol solvate (Iwasaki *et al.*, 1993). Tablet and dry-syrup formulations using form II crystals are in clinical use (Yajima *et al.*, 1999, 2002). CAM tablets are stable in an acidic solution such as gastric fluid, despite the observation that CAM molecules decompose rapidly under low pH conditions (Nakagawa *et al.*, 1992). This is because, in acidic solution, a gel of CAM is formed on the surface of the tablets and this gel prevents the acidic solution from permeating into the tablets (Fujiki *et al.*, 2011). Thus, tablets of acid-labile CAM can be administered orally without an enteric coating. Single-crystal structures of forms 0 (Jin *et al.*, 2009), II (Stephenson *et al.*, 1997) and III (Liang & Yao, 2008) and of the hydrochloride salt and the methanol solvate have been reported. However, the crystal structure of form I remains unknown because of the difficulties associated with the preparation of single crystals suitable for X-ray analysis. Form I, a metastable form, is prepared by drying form 0 in a vacuum at 273–323 K or by exposing form 0 to air at room temperature (Liu *et al.*, 1999). Form I shows an endothermic peak at around 413 K, as determined by differential thermal analysis, and transforms to form II (Tozuka *et al.*, 2002). Form I has an intrinsic rate of dissolution about three times that of form II (Liu *et al.*, 1999) and this higher dissolution rate suggests the potential application of form I in a new drug formulation. We report here the crystal structure of CAM form I determined by the X-ray powder diffraction method using synchrotron radiation.



CAM

The structure determination of CAM form I by direct methods was initially attempted but was unsuccessful. This is probably because the quality of the extracted diffraction intensities at the higher 2θ angles was not sufficiently high for

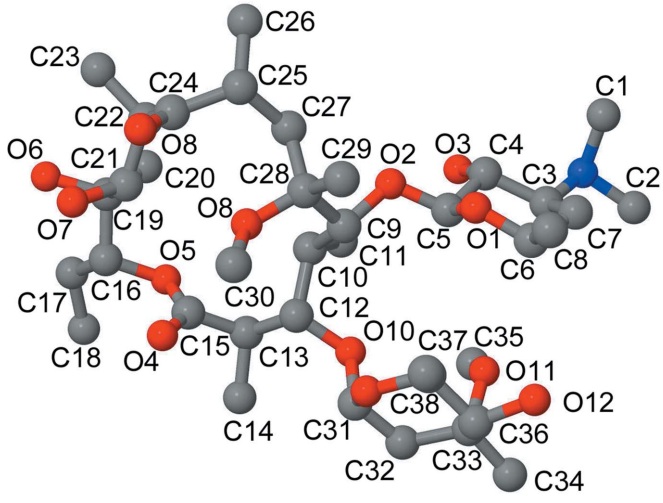


Figure 1
The molecular structure of CAM form I. H atoms have been omitted for clarity. The radii of the spheres are arbitrary.

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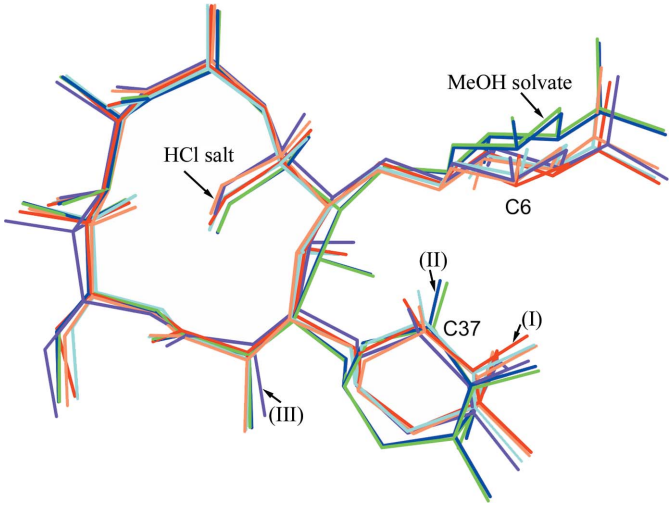

Figure 2
Superposition of the structures of CAM forms I (red in the electronic version of the paper), 0 (cyan), II (blue) and III (purple), the hydrochloride salt (coral) and the methanol solvate (green).

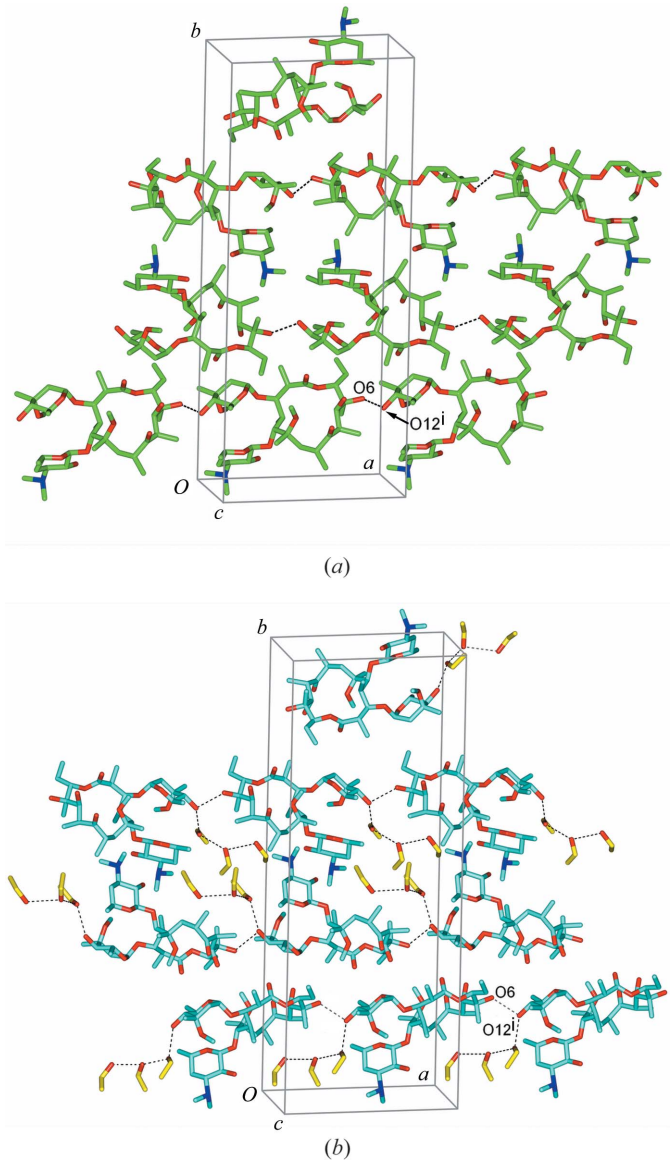

Figure 3
The crystal packing of (*a*) CAM form I (this study) and (*b*) CAM form 0 [Tian *et al.*, 2011; space group $P2_12_12_1$, a = 14.5980 (16), b = 38.368 (4) and c = 8.7685 (9) Å]. Hydrogen bonds are drawn as dotted lines. The unit cell is drawn with thick grey lines. (In the electronic version of the paper, the N and O atoms are shown in blue and red, respectively, the C atoms of CAM in forms I and 0 are shown in green and cyan, respectively, and the C atoms of the ethanol solvent molecules in form 0 are shown in yellow.) [Symmetry code: (i) $x+1, y, z$.]

structure determination by direct methods, owing to the severe overlap of the diffraction peaks caused by the long cell parameters of form I, even though highly parallel synchrotron X-ray radiation was used. The reported crystal structures of CAM share a common overall conformation, suggesting that the CAM molecule is rather rigid. Therefore, structure determination by the molecular replacement method was performed, which gave a clear solution and resulted in the determination of the CAM form I crystal structure after refinement by the Rietveld method (Fig. 1). The molecular replacement method has been used primarily for the structure determination of single crystals of biomacromolecules such as proteins that contain a very large number of non-H atoms, because the method can considerably reduce the calculation time for structure determination (Rossmann, 2001). The structure determination of CAM form I demonstrates that the molecular replacement method is a suitable alternative for the determination of an initial structure for Rietveld refinement, especially when the asymmetric unit of the crystal structure contains a large number of non-H atoms.

In the crystal structure of form I, a void space is found near the crystallographic twofold axis. The fractional coordinates of its centre of gravity are (0.000, 0.093, 0.548) and its volume is 36 (2) Å^3, as calculated by *PLATON* (Spek, 2009). The void space is surrounded by five methyl groups of the desosamine (C2 and C8), cladinose (C34 and C35) and aglycone rings (C23), indicating that the space is hydrophobic. The volume of the space is too small to accommodate an ethanol molecule, and the difference Fourier maps show no positive density features in this space. These observations indicate the absence of a solvent molecule in the crystal structure. This is consistent with the results of the thermogravimetric analysis, which showed that the powder of CAM form I crystals lost 0.3% of its weight, which is negligible compared with the weight of an ethanol molecule (6.0%). The hydrophobic void space in form I contrasts with the void space observed in form II, which is able to accommodate a water molecule *via* a hydrogen bond (Tian *et al.*, 2009).

Fig. 2 shows the superimposed CAM structures. The positional r.m.s. differences of all non-H atoms between CAM forms I and 0, forms I and II, forms I and III, form I and the hydrochloride salt, and form I and the methanol solvate are 0.22, 0.60, 0.49, 0.29 and 0.67 Å, respectively. The largest deviations are observed at the cladinose and desosamine rings. The separation between the two rings, represented by the distance between atoms C6 and C37, is smallest in form I at 3.69 (5) Å, and those in forms 0 and III and the hydrochloride

salt are comparable or marginally wider [3.813 (4), 3.833 (4) and 3.753 (6) Å, respectively]. In contrast with these observations, the separations in form II and the methanol solvate are greater by approximately 0.5 Å [4.226 (4) and 4.280 (5) Å, respectively].

In the crystal structure of form I, an intermolecular hydrogen bond is formed between atom O6 of the aglycone ring of the molecule at (x, y, z) and atom O12 of the cladinose ring of the molecule at ($x+1$, y, z), with an O$\cdots$O distance of 2.67 (4) Å, as shown in Fig. 3(*a*). The CAM molecules are aligned in a head-to-tail manner parallel to the *a* axis *via* these intermolecular hydrogen bonds. The dimethylamine groups of the desosamine rings interdigitate between neighbouring head-to-tail CAM alignments. The methyl groups (C2) of the dimethylamine groups, related by a crystallographic twofold axis, are in van der Waals contact, at a distance of 3.34 (4) Å.

This novel molecular packing of CAM found in form I provides insights into a mechanism that describes the easy transformation from form 0 to form I. In form 0, the head-to-tail arrangement of the CAM molecules with hydrogen bonds between atoms O6 and O12 is also parallel along the *a* axis, as shown in Fig. 3(*b*). The large channels parallel to the *c* axis are located near the dimethylamine groups and the channels are filled with ethanol solvent molecules. When form 0 crystals are dried under a vacuum, the ethanol molecules evaporate and the channels become vacant. To fill the vacant channels with dimethylamine groups, the head-to-tail CAM alignments shift by approximately *a*/2, 2 Å and *c*/2 along the *a*, *b* and *c* axes, respectively, resulting in the transformation of form 0 to form I with a decrease in the cell volume of 11%, although the small void space remains near the dimethylamine groups, as described above.

Intermolecular hydrogen bonds between atoms O6 and O12 are also formed in form II. However, the CAM molecules are arranged not linearly, as in the case of forms I and 0, but in a zigzag arrangement (Tian *et al.*, 2009). The differences in the CAM molecular arrangements in these crystals indicate that a greater rearrangement of the CAM molecules needs to occur in the transformation from form I to form II. This may explain why the transformation from form I to form II proceeds at an elevated temperature of 413 K, whereas the transformation from form 0 to form I proceeds at ambient temperature.

Experimental

Bulk CAM (purity >99%) was purchased from Shiono Chemical Co. Ltd (Tokyo, Japan). CAM (1.8 g) was mixed with ethanol (60 ml) and completely dissolved by warming to 343 K. The CAM solution was cooled rapidly to room temperature with vigorous stirring in an ice-cold water bath and then stirred continuously for 3 h at room temperature. The fine powder of CAM form 0 crystals thus obtained was collected by filtration and completely dried over a period of 24 h in a vacuum at room temperature in order to transform the crystals into form I. To quantify the volatiles in the crystal, thermogravimetric analysis was performed over the temperature range 303–503 K using a TG/DTA320 instrument (SEIKO Instruments Inc., Tokyo, Japan) at a heating rate of 5 K min^{-1} and an air flow rate of 100 ml min^{-1}.

Crystal data

$C_{38}H_{69}NO_{13}$
M_r = 748.0
Orthorhombic, $P2_12_12$
a = 14.4531 (4) Å
b = 34.6885 (8) Å
c = 8.7114 (2) Å
V = 4367.52 (19) Å^3
Z = 4
Synchrotron radiation, λ = 1.3000 Å
μ = 0.42 mm^{-1}
T = 300 K
Cylinder, 3.0 × 0.3 mm

Data collection

Debye-Scherrer camera diffractometer
Specimen mounting: capillary
Data collection mode: transmission
Scan method: stationary detector
$2\theta_{fixed}$ = 65

Refinement

R_p = 0.038
R_{wp} = 0.057
R_{exp} = 0.015
R_{Bragg} = 0.047
$R(F)$ = 0.042
$R(F^2)$ = 0.04212
χ^2 = 13.712
6201 data points
185 parameters
96 restraints
H-atom parameters constrained

The powder of the form I crystals was enclosed in a 0.3 mm Lindemann glass capillary. The X-ray powder diffraction data were collected at SPring-8 BL19B2 (Osaka *et al.*, 2010), which is equipped with a high-resolution type Debye–Scherrer camera and a curved imaging-plate detector (Takata *et al.*, 2002). The wavelength was set at 1.3000 (1) Å. During data collection, the sample was kept at 300 K and rotated at 1 r min^{-1} to reduce possible preferential orientation.

Processing the powder data of CAM form I crystals with *N-TREOR09* (Altomare, Campi *et al.*, 2009) implemented in *EXPO2009* (Altomare, Camalli *et al.*, 2009) suggested a primitive orthorhombic crystal system with the lattice parameters a = 14.452, b = 34.689 and c = 8.712 Å. Although a 001 reflection was observed clearly, it was unclear whether h00 and 0k0 reflections violated the extinction rule or not, because of the overlaps of the reflection peaks. The intensities of the reflections were extracted from the powder diffraction data assuming a primitive orthorhombic crystal system using *EXPO2009*.

Structure determination by the molecular replacement method was performed using reflections between 17.3–3.0 Å d-spacings using *MOLREP* implemented in *CCP4* (Collaborative Computational Project, Number 4, 1994). The search model was the single-crystal structure of CAM form 0, which was refined at high resolution ($1.49 < \theta < 27.12^\circ$, Mo $K\alpha$; Tian *et al.*, 2011). The overall isotropic atomic displacement parameter was fixed at 0.063 Å^2 and the H atoms were excluded. A clear solution without any inappropriate intermolecular atomic contacts in the crystal structure was found only when the space group was assumed to be $P2_12_12$. Structure determination by the direct space technique using simulated annealing gave the same solution in the case of the space group $P2_12_12$, and no plausible solution was obtained when assuming other primitive orthorhombic space groups. The bond lengths and angles were restrained to those of the CAM form 0 crystal structure and the positional parameters of the atoms were refined using *REFMAC* (Murshudov *et al.*, 1997). The crystallographic R factor converged to 0.187 for reflections between 17.3 and 2.3 Å d-spacings. The partially refined structure was the starting model for Rietveld refinement using *RIETAN-FP* (Izumi & Momma, 2007). The split pseudo-Voigt function was used to fit the peaks in the powder diffraction pattern, and the background was modelled by Legendre polynomials. The geometry of the CAM molecule was restrained as described above. The overall atomic displacement parameter was applied to all atoms, including the H atoms, and was refined isotropically, and the March–Dollase function

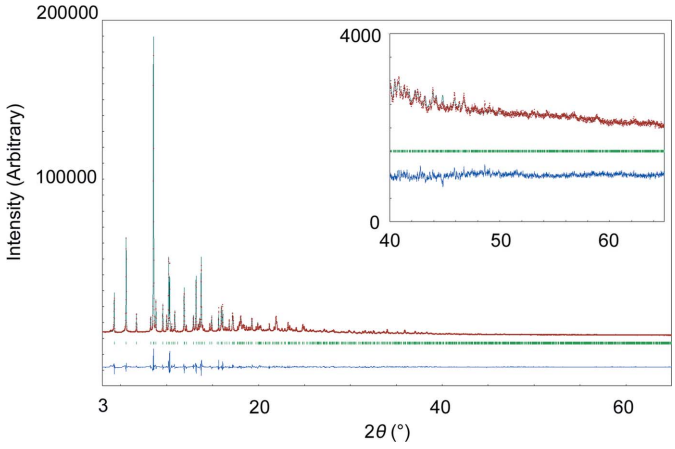

Figure 4
The final Rietveld plot of CAM form I. The experimental diffraction profile is indicated by crosses (red in the electronic version of the paper). The calculated diffraction profile is shown as the top solid line (green), the difference profile is shown as the bottom solid line (blue) and the vertical bars (green) between the solid lines correspond to the positions of the Bragg peaks.

(Dollase, 1986) was used for correction of the preferred orientation in the (010) plane. Before the final refinement, H atoms were generated at their theoretical positions using *EXPO2009* and *Jmol* (Hanson, 2010), and were refined as riding, with C—H = 0.96 Å and O—H = 0.82 Å. At the end of the refinement, the r.m.s. differences of the bond lengths and angles from their target values were 0.022 Å and 2.51°, respectively. Fig. 4 shows the observed, calculated and difference patterns after the Rietveld refinement. A small unassigned peak was observed at $2\theta = 3.89°$. This peak is thought to be derived from a trace quantity of form 0 crystals that remain in the CAM form I sample, because the 2θ value is identical to that of the 020 peak of form 0; its diffraction intensity is one of the strongest in the form 0 crystal structure.

Data collection: local software (Takata *et al.*, 2002); cell refinement: *EXPO2009* (Altomare, Camalli *et al.*, 2009) and *RIETAN-FP* (Izumi & Momma, 2007); data reduction: local software (Takata *et al.*, 2002); program(s) used to solve structure: *CCP4* (Collaborative Computational Project, Number 4, 1994); program(s) used to refine structure: *RIETAN-FP*; molecular graphics: *CCP4MG* (McNicholas *et al.*, 2011); software used to prepare material for publication: *publCIF* (Westrip, 2010).

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: CU3008). Services for accessing these data are described at the back of the journal.

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