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# The antitumour drug 7-ethyl-10-hydroxycamptothecin monohydrate and its solid-state hydrolysis mechanism on heating

**Md. Ashraf Ali, Shuji Noguchi, Miteki Watanabe, Yasunori Iwao and Shigeru Itai**

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# The antitumour drug 7-ethyl-10-hydroxycamptothecin monohydrate and its solid-state hydrolysis mechanism on heating

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**Keywords:** 7-ethyl-10-hydroxycamptothecin; antitumour drugs; topoisomerase I inhibitor; solid-state hydrolysis; crystal structure; anti-cancer agent; irinotecan; synchrotron.

**CCDC reference:** 1504071

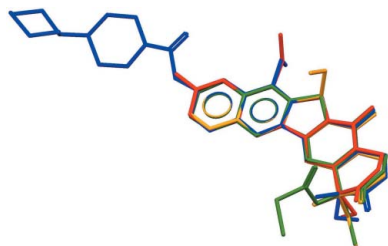
**Supporting information:** this article has supporting information at journals.iucr.org/c

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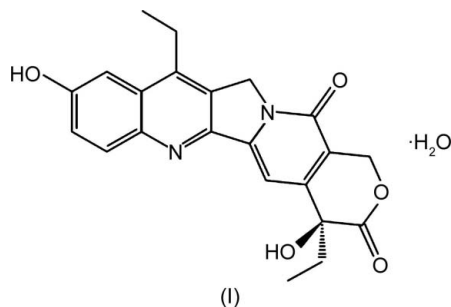
7-Ethyl-10-hydroxycamptothecin [systematic name: (4*S*)-4,11-diethyl-4,9-dihydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione, SN-38] is an antitumour drug which exerts activity through the inhibition of topoisomerase I. The crystal structure of SN-38 as the monohydrate, C<sub>22</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>·H<sub>2</sub>O, reveals that it is a monoclinic crystal, with one SN-38 molecule and one water molecule in the asymmetric unit. When the crystal is heated to 473 K, approximately 30% of SN-38 is hydrolyzed at its lactone ring, resulting in the formation of the inactive carboxylate form. The molecular arrangement around the water molecule and the lactone ring of SN-38 in the crystal structure suggests that SN-38 is hydrolyzed by the water molecule at (*x*, *y*, *z*) nucleophilically attacking the carbonyl C atom of the lactone ring at (*x* − 1, *y*, *z* − 1). Hydrogen bonding around the water molecules and the lactone ring appears to promote this hydrolysis reaction: two carbonyl O atoms, which are hydrogen bonded as hydrogen-bond acceptors to the water molecule at (*x*, *y*, *z*), might enhance the nucleophilicity of this water molecule, while the water molecule at (−*x*, *y* +  $\frac{1}{2}$ , −*z*), which is hydrogen bonded as a hydrogen-bond donor to the carbonyl O atom at (*x* − 1, *y*, *z* − 1), might enhance the electrophilicity of the carbonyl C atom.

## 1. Introduction

7-Ethyl-10-hydroxycamptothecin [systematic name: (4*S*)-4,11-diethyl-4,9-dihydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione, SN-38] is an active metabolite of the prodrug irinotecan. SN-38 is an effective anticancer agent, which induces cell death by inhibiting the nuclear enzyme topoisomerase I during DNA replication and transcription (Pommier, 2006). Although the prodrug irinotecan has been used widely for the treatment of colorectal cancer and many other solid tumours (Potmesil, 1994), it has been reported that only 2–8% of an injected dose of irinotecan in humans was converted into active SN-38 (Rothenberg *et al.*, 2001). SN-38 has been reported to show a 10<sup>2</sup>- to 10<sup>3</sup>-fold more potent cytotoxicity *in vitro* compared with irinotecan (Chabot, 1997). Various types of formulations and drug-delivery systems have been investigated for the formulation of SN-38 to improve its applicability by increasing water solubility, bioavailability and stability. Examples include chemical modification using polymers (Sapra *et al.*, 2008; Kolhatkar *et al.*, 2008), and nanoparticle formulations such as liposomes (Zhang *et al.*, 2004), micelles (Hamaguchi *et al.*, 2010), poly



lactic-co-glycolic acid nanoparticles (Sepehri *et al.*, 2014) and cubosomes (Ali *et al.*, 2016). SN-38 exists in solution in an equilibrium between the lactone form and the hydrolyzed carboxylate form, which is inactive (Thakur *et al.*, 2010). Although the crystal structure of irinotecan has been determined using single-crystal X-ray diffraction [Cambridge Structural Database (CSD; Groom & Allen, 2014) entry VOGCAV; Sawada *et al.*, 1991], that of SN-38 has not yet been reported. In this work, we have determined the single-crystal structure of SN-38 monohydrate, (I), and have also investigated its solid-state hydrolysis on heating.



## 2. Experimental

### 2.1. Crystallization

SN-38 was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). All other reagents used in this study were of the highest grade commercially available. SN-38 was dissolved in acetone, and the acetone solution was centrifuged at 10 000 *g* for 10 min. The supernatant (approximately 1 ml) was poured into a Petri dish and allowed to evaporate slowly at room temperature. Fine transparent crystals of (I) with a columnar shape had grown within 1 d. The powder X-ray diffraction (PXRD) pattern of the recrystallized crystals measured using a Mini Flex II (Rigaku Corporation, Tokyo, Japan) diffractometer was identical to that of the SN-38 crystals purchased from Tokyo Chemical Industry. This ensured that the crystal forms were the same before and after the recrystallization process. Interestingly, the supplied material is not labelled as the monohydrate, even though our experiments showed that the newly purchased unopened reagent was pure monohydrate.

### 2.2. Refinement

Crystal data, data collection and structure refinement results are summarized in Table 1. All H atoms, including those in the water molecule, were located in a difference Fourier map. The positions of the water H atoms were refined with O—H and H $\cdots$ H distance restraints of 0.84 (1) and 1.34 (1) Å, respectively. All other H atoms were placed in idealized positions and constrained to ride on their parent atoms, with C—H = 0.95–0.99 Å and O—H = 0.84 Å. For all H atoms,  $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{C}, \text{O})$ .

### 2.3. PXRD measurement on heating

Fine crystal powders of SN-38 were prepared using a pestle and mortar. The ground crystal powders were packed into

**Table 1**  
Experimental details.

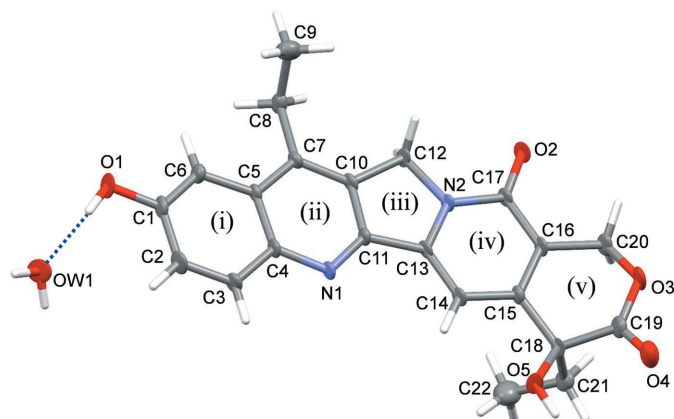
|   |   |
|---|---|
| Crystal data  |   |
| Chemical formula  | C <sub>22</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> ·H <sub>2</sub> O |
| $M_r$   | 410.42  |
| Crystal system, space group   | Monoclinic, $P2_1$  |
| Temperature (K)   | 100   |
| $a, b, c$ (Å)   | 8.529 (2), 7.352 (2), 15.075 (3)  |
| $\beta$ (°)   | 100.18 (3)  |
| $V$ (Å <sup>3</sup> )   | 930.3 (4)   |
| $Z$   | 2   |
| Radiation type  | Synchrotron (SPring-8 BL02B1),<br>$\lambda = 0.70041$ Å                         |
| $\mu$ (mm <sup>-1</sup> )   | 0.11  |
| Crystal size (mm)   | 0.20 × 0.01 × 0.01  |
| Data collection   |   |
| Diffractometer  | Rigaku Mercury2 four-circle<br>diffractometer with CCD area<br>detector         |
| No. of measured, independent and<br>observed [ $I > 2\sigma(I)$ ] reflections | 13173, 4345, 3684   |
| $R_{\text{int}}$  | 0.056   |
| $(\sin \theta/\lambda)_{\text{max}}$ (Å <sup>-1</sup> )                       | 0.685   |
| Refinement  |   |
| $R[F^2 > 2\sigma(F^2)]$ , $wR(F^2)$ , $S$                                     | 0.043, 0.120, 0.84  |
| No. of reflections  | 4345  |
| No. of parameters   | 281   |
| No. of restraints   | 16  |
| H-atom treatment  | H atoms treated by a mixture of<br>independent and constrained<br>refinement    |
| $\Delta\rho_{\text{max}}$ , $\Delta\rho_{\text{min}}$ (e Å <sup>-3</sup> )    | 0.42, -0.28   |

Computer programs: *RAPID-AUTO* (Rigaku, 1999), *SHELXT* (Sheldrick, 2015a), *SHELXL2014* (Sheldrick, 2015b), *SHELXLE* (Hübschle *et al.*, 2011), *Mercury* (Macrae *et al.*, 2008) and *pubCIF* (Westrip, 2010).

Lindemann glass capillaries of 0.3 mm diameter and used for a PXRD study of the effect of heating. PXRD data were collected on beamline BL5S2 at the Aichi Synchrotron Radiation Center, which was equipped with a Debye–Sherrer camera and a Pilatus 100K area detector (Rigaku, Tokyo, Japan). The distance between the detector and the sample was set to 340 mm and the detector was tilted by  $2\theta = 9^\circ$ , so that powder diffractions with  $2\theta$  values of up to  $16^\circ$  could be recorded. The wavelength was set to 1.000 Å. During data collection, the samples were rotated at 3.3 r min<sup>-1</sup> to minimize the effect of preferential orientation. The samples were heated from 298 to 573 K at a rate of 10 K min<sup>-1</sup> using an N<sub>2</sub> gas flow. PXRD data were recorded sequentially with an exposure time of 18 s. The amount of crystals upon heating was estimated using the integrated intensities,  $I(T)$ , of the diffraction peaks at temperature  $T$ , and changes were analysed by plotting the ratio  $I(T)/I_{\text{max}}$ , where  $I_{\text{max}}$  is the maximum value of  $I(T)$ . The peak used for the plot was that at a  $2\theta$  value of around  $7.2^\circ$  [ $(\bar{1}01)$ ] in the case of the SN-38·H<sub>2</sub>O crystals, while that at a  $2\theta$  value of around  $10.1^\circ$  was used in the case of the crystal form which emerged on heating.

### 2.4. High-performance liquid chromatography (HPLC) analyses

After heating to 473 K for 10 min, the SN-38·H<sub>2</sub>O samples were dissolved in acetonitrile and analysed using HPLC to



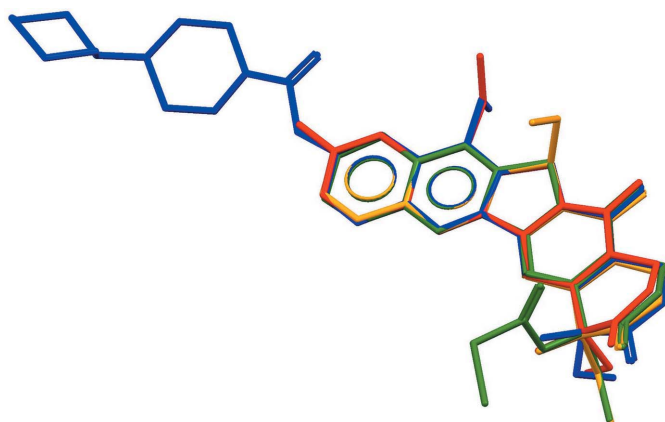
**Figure 1**

The molecular structure of SN-38 monohydrate and the atom-numbering scheme used. C, H, O and N atoms are shown in grey, white, red and pale violet, respectively. Displacement ellipsoids are drawn at the 75% probability level. Roman numerals in parentheses indicate the ring-numbering system used. The hydrogen bond between SN-38 and the water molecule is shown as a blue dotted line.

identify the products on heating, under the conditions described previously (Ali *et al.*, 2016). A TSKgel ODS-80Tm column was employed and the mobile phase used was a 1:1 (*v/v*) mixture of acetonitrile and 25 mM sodium phosphate buffer (pH 7.4). SN-38 and degradation products on heating were detected with a UV absorption spectrometer set at a wavelength of 265 nm.

### 3. Results and discussion

The refined crystal structure reveals that the asymmetric unit of (I) consists of one SN-38 molecule and one water molecule (Fig. 1), indicating that the crystal is SN-38 monohydrate. The water molecule may be a by-product generated during the SN-38 synthesis (Latinen, 2006). Rings (i), (ii), (iii) and (iv) of SN-38 (defined in Fig. 1) are almost coplanar but slightly curved, and the angle formed by the best-fit planes of rings (i) and (iv) is  $6.89 (14)^\circ$ . When the aromatic rings (i), (ii) and (iii)

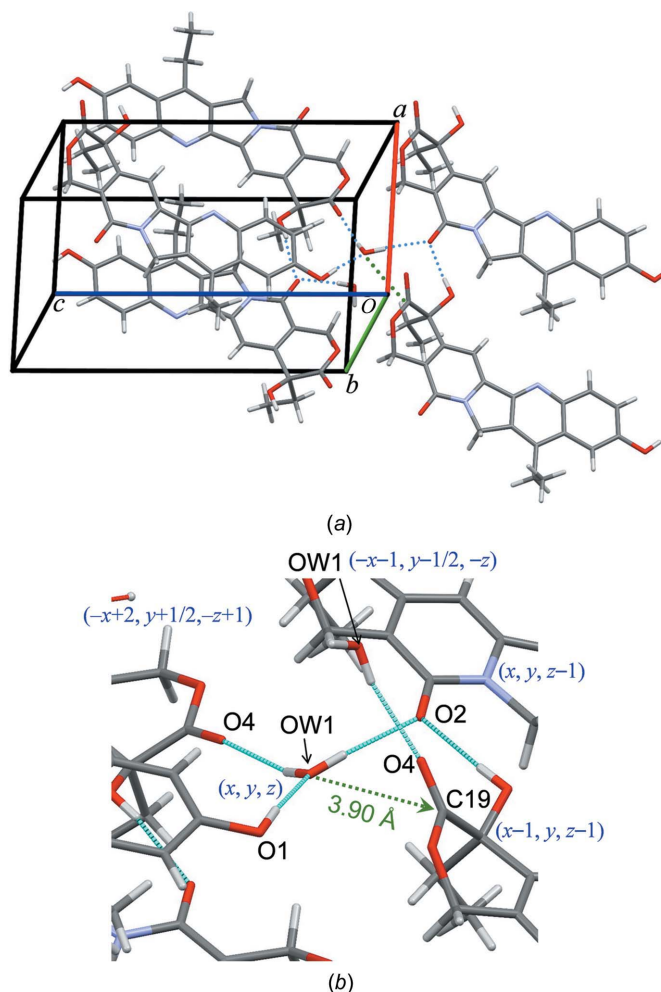


**Figure 2**

Superposition of the molecular structures of SN-38 (red), irinotecan (blue), camptothecin iodoacetate (green) and  $5\beta$ -(hydroxymethyl)-camptothecin (orange). H atoms have been omitted for clarity.

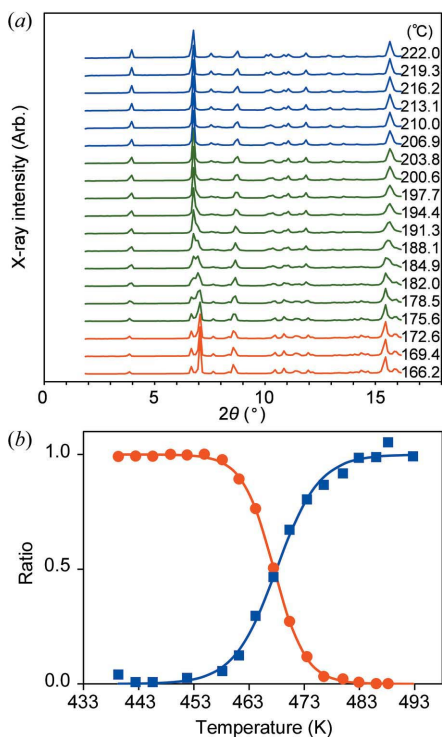
of SN-38 are superimposed on those of irinotecan, camptothecin iodoacetate (CSD entry CAMPTC10; McPhail & Sim, 1968) and  $5\beta$ -(hydroxymethyl)camptothecin (CSD entry YIZYEL; Wang *et al.*, 1995), a difference in conformation is found at the lactone rings (Fig. 2). All the lactone rings of these compounds adopt a boat conformation, as is often observed in the six-membered lactone rings of multicyclic compounds (Dai *et al.*, 2010; Jenkinson *et al.*, 2010). The C15—C16—C20—O3 torsion angle in SN-38, which indicates the planarity of the six-membered lactone ring, is  $-30.0 (4)^\circ$ , while those of irinotecan, camptothecin iodoacetate and  $5\beta$ -(hydroxymethyl)camptothecin are  $-14 (2)$ ,  $-33.3$  and  $-24.9^\circ$ , respectively. This shows that the lactone rings of SN-38, camptothecin iodoacetate and  $5\beta$ -(hydroxymethyl)camptothecin are less planar than that of irinotecan. This variation in conformation might suggest conformational flexibility of the lactone rings in the camptothecin skeleton.

In the crystal structure of (I), aromatic rings (i)–(iv) of SN-38 are nearly parallel to the *ac* plane and are stacked along



**Figure 3**

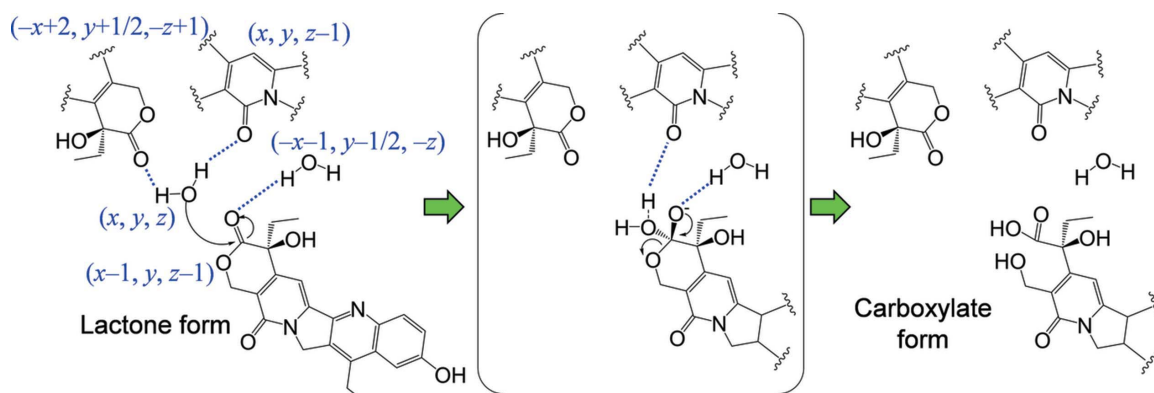
(a) The crystal packing of SN-38 monohydrate. Hydrogen bonds are shown as blue dotted lines. The O atom of the water molecule and a carbonyl C atom in the lactone ring nearest to the water molecule are connected with a green dotted line. (b) The hydrogen-bond network around the water molecule.



**Figure 4**  
 (a) PXRD profiles of SN-38 monohydrate on heating. Enlarged profiles around  $10.1^\circ$  are shown in supplementary Fig. S1. (b) Changes in the diffraction intensity on heating.  $I(T)/I_{\max}$  values are plotted as a function of temperature  $T$  for the  $(\bar{1}01)$  diffraction peak of the SN-38 monohydrate crystal (red) and that at a  $2\theta$  value of around  $10.1^\circ$  (blue). The equation for a sigmoidal curve,  $I(T)/I_{\max} = \{1 + \exp[-k(T - C)]\}^{-1} + A$ , was fitted to the plots using a non-linear least-squares method to determine the parameters  $A$ ,  $C$  and  $k$  (Watanabe *et al.*, 2016), shown as red and blue solid lines.

the  $b$ -axis direction (Fig. 3a). Four hydrogen bonds are found in the crystal structure: one between neighbouring SN-38 molecules, and the rest between SN-38 and water molecules (Table 2). The hydrogen bond between the O5–H group at  $(x, y, z)$  and atom O2 at  $(x + 1, y, z)$  links the SN-38 molecules along the  $a$  axis.

When the SN-38·H<sub>2</sub>O crystals were heated, the intensities of the diffraction peaks of the SN-38·H<sub>2</sub>O crystals began to



**Figure 5**  
 A possible mechanism for the hydrolytic degradation of SN-38 in the crystal on heating.

**Table 2**  
 Hydrogen-bond geometry ( $\text{\AA}$ ,  $^\circ$ ).

| $D-H \cdots A$                      | $D-H$    | $H \cdots A$ | $D \cdots A$ | $D-H \cdots A$ |
|-------------------------------------|----------|--------------|--------------|----------------|
| O1–H1 $\cdots$ O2 <sup>i</sup>      | 0.84     | 1.91         | 2.732 (3)    | 168            |
| O5–H5 $\cdots$ O2 <sup>i</sup>      | 0.84     | 2.06         | 2.896 (3)    | 173            |
| OW1–HW1A $\cdots$ O2 <sup>ii</sup>  | 0.85 (1) | 2.07 (2)     | 2.881 (3)    | 160 (4)        |
| OW1–HW1B $\cdots$ O4 <sup>iii</sup> | 0.84 (1) | 2.13 (2)     | 2.894 (3)    | 152 (4)        |

Symmetry codes: (i)  $x, y, z + 1$ ; (ii)  $x - 1, y, z$ ; (iii)  $-x + 1, y - \frac{1}{2}, -z + 2$ .

decrease at 453 K and became almost zero at around 480 K (Fig. 4a). Instead, new diffraction peaks emerged at 453 K and their diffraction intensities reached a constant value beyond 480 K. The temperature at which the intensities of the  $(\bar{1}01)$  peaks halved was 467 K, as calculated using a fitted sigmoidal curve (Fig. 4b). These changes in the PXRD profiles indicate that the SN-38 monohydrate crystal is transformed into another crystal form by heating. Cell parameters regarding the peaks found in the PXRD profiles above 478 K could not be determined using *EXPO2014* (Altomare *et al.*, 2013), probably because the sample was a mixture of polymorphs and degradation products of SN-38. HPLC analyses of the SN-38·H<sub>2</sub>O crystals that were heated to 473 K for 10 min revealed that the sample consisted of intact SN-38 (60%), the carboxylate form of SN-38 (30%) and unknown compounds (10%). This indicates that 60% of SN-38 was transformed into another polymorph, possibly an anhydrate form, while 30% was hydrolysed and 10% was degraded as a result of heating. The sum of the ratios in Fig. 4(b) was almost 1 during the heating process, suggesting that the proportion of SN-38 monohydrate decreased and the compound relating to the reflection around  $10.1^\circ$  emerged simultaneously.

In the crystal structure of (I), the O atom of the water molecule, OW1, is located at a distance of 3.897 (3)  $\text{\AA}$  from the carbonyl C19 atom of the lactone ring at  $(x - 1, y, z - 1)$ , and the angle formed by the OW1 $\cdots$ C19 line and the best-fit plane of the carbonyl group of the lactone is  $54.9^\circ$ . This suggests that the water molecule can get close to atom C19 at  $(x - 1, y, z - 1)$  in the crystal structure. The O4 atom of the lactone ring at  $(x - 1, y, z - 1)$  is hydrogen bonded as a hydrogen-bond acceptor to the water molecule at  $(-x - 1, y - \frac{1}{2}, -z)$ . These molecular arrangements in the crystal structure imply that the

lactone ring of SN-38 may become hydrolysed, as shown in Fig. 5.

First, the water molecule at  $(x, y, z)$  would nucleophilically attack the carbonyl C19 atom at  $(x - 1, y, z - 1)$ . The hydrogen bond between atom HW1A of the water molecule and the carbonyl O2 atom at  $(x, y, z - 1)$  would enhance the nucleophilicity of the water molecule as a general base, while the hydrogen bond between the carbonyl O4 atom at  $(x - 1, y, z - 1)$  as a hydrogen-bond acceptor and the water molecule at  $(-x - 1, y - \frac{1}{2}, -z)$  would enhance the electrophilicity of C19. A tetrahedral intermediate would then be formed by the nucleophilic attack of the water molecule, and the intermediate might also be stabilized through these hydrogen bonds. Atom O3 in the tetrahedral intermediate would leave C19, finally resulting in the formation of the carboxylate form of SN-38. Once the carboxylate form has formed, the molecular arrangement conducive to hydrolysis of the lactone ring may be disturbed in the crystal structure, which may partly explain why not all SN-38 molecules in the crystal were hydrolysed on heating.

These heat-labile characteristics of SN-38 monohydrate suggest that heating processes should be carefully avoided in the manufacture of formulations containing the monohydrate.

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## supporting information

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## The antitumour drug 7-ethyl-10-hydroxycamptothecin monohydrate and its solid-state hydrolysis mechanism on heating

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### Computing details

Data collection: *RAPID-AUTO* (Rigaku, 1999); cell refinement: *RAPID-AUTO* (Rigaku, 1999); data reduction: *RAPID-AUTO* (Rigaku, 1999); program(s) used to solve structure: *SHELXT* (Sheldrick, 2015a); program(s) used to refine structure: *SHELXL2014/7* (Sheldrick, 2015b), *SHELXL* (Hübschle *et al.*, 2011); molecular graphics: *Mercury* (Macrae *et al.*, 2008); software used to prepare material for publication: *publCIF* (Westrip, 2010).

(4S)-4,11-Diethyl-4,9-dihydroxy-1H-pyrano[3',4':6,7] indolizino[1,2-b]quinoline-3,14(4H,12H)dione

### Crystal data

$C_{22}H_{20}N_2O_5 \cdot H_2O$

$M_r = 410.41$

Monoclinic,  $P2_1$

$a = 8.529$  (2) Å

$b = 7.352$  (2) Å

$c = 15.075$  (3) Å

$\beta = 100.18$  (3)°

$V = 930.3$  (4) Å<sup>3</sup>

$Z = 2$

$F(000) = 432$

$D_x = 1.465$  Mg m<sup>-3</sup>

Synchrotron (SPring-8 BL02B1) radiation,  $\lambda = 0.70041$  Å

Cell parameters from 255 reflections

$\theta = 1.2$ – $6.7^\circ$

$\mu = 0.11$  mm<sup>-1</sup>

$T = 100$  K

Column, pale yellow

$0.20 \times 0.01 \times 0.01$  mm

### Data collection

Rigaku Mercury2 four-circle diffractometer with CCD area detector

Radiation source: synchrotron

$\omega$  scan

13173 measured reflections

4345 independent reflections

3684 reflections with  $I > 2\sigma(I)$

$R_{int} = 0.056$

$\theta_{max} = 28.7^\circ$ ,  $\theta_{min} = 1.4^\circ$

$h = -11 \rightarrow 8$

$k = -6 \rightarrow 9$

$l = -20 \rightarrow 20$

### Refinement

Refinement on  $F^2$

Least-squares matrix: full

$R[F^2 > 2\sigma(F^2)] = 0.043$

$wR(F^2) = 0.120$

$S = 0.84$

4345 reflections

281 parameters

16 restraints

Primary atom site location: structure-invariant direct methods

Secondary atom site location: difference Fourier map

Hydrogen site location: mixed

H atoms treated by a mixture of independent and constrained refinement

$w = 1/[\sigma^2(F_o^2) + (0.1P)^2]$   
where  $P = (F_o^2 + 2F_c^2)/3$

$(\Delta/\sigma)_{max} < 0.001$

$\Delta\rho_{max} = 0.42$  e Å<sup>-3</sup>

$\Delta\rho_{min} = -0.28$  e Å<sup>-3</sup>



*Special details*

**Geometry.** All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.

*Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ( $\text{\AA}^2$ )*

|      | <i>x</i>   | <i>y</i>   | <i>z</i>     | $U_{\text{iso}}^*/U_{\text{eq}}$ |
|------|------------|------------|--------------|----------------------------------|
| O1   | 0.3063 (2) | 0.4942 (3) | 0.14614 (11) | 0.0191 (5)                       |
| H1   | 0.3636     | 0.4929     | 0.1062       | 0.023*                           |
| N1   | 0.6918 (2) | 0.4835 (3) | 0.48097 (12) | 0.0109 (4)                       |
| C1   | 0.4001 (3) | 0.4971 (4) | 0.22858 (15) | 0.0136 (5)                       |
| O2   | 0.5040 (2) | 0.4379 (3) | 0.83245 (11) | 0.0148 (4)                       |
| N2   | 0.5875 (2) | 0.4670 (3) | 0.69791 (12) | 0.0101 (4)                       |
| C2   | 0.5673 (3) | 0.4941 (5) | 0.23798 (15) | 0.0159 (6)                       |
| H2   | 0.6159     | 0.4925     | 0.1859       | 0.019*                           |
| C3   | 0.6596 (3) | 0.4934 (4) | 0.32162 (15) | 0.0138 (5)                       |
| H3   | 0.7723     | 0.4927     | 0.3272       | 0.017*                           |
| O3   | 0.9750 (2) | 0.2875 (3) | 0.94861 (12) | 0.0178 (4)                       |
| C4   | 0.5905 (3) | 0.4938 (4) | 0.40030 (14) | 0.0109 (5)                       |
| O4   | 1.2123 (2) | 0.2186 (3) | 0.92289 (13) | 0.0216 (5)                       |
| C5   | 0.4212 (3) | 0.5057 (4) | 0.39111 (14) | 0.0099 (5)                       |
| O5   | 1.1680 (2) | 0.3924 (3) | 0.76177 (11) | 0.0150 (5)                       |
| H5   | 1.2640     | 0.4005     | 0.7866       | 0.018*                           |
| OW1  | 0.4846 (2) | 0.5435 (4) | 0.01413 (13) | 0.0259 (6)                       |
| HW1A | 0.488 (4)  | 0.500 (5)  | -0.0369 (14) | 0.031*                           |
| HW1B | 0.562 (3)  | 0.614 (5)  | 0.027 (2)    | 0.031*                           |
| C6   | 0.3287 (3) | 0.5077 (4) | 0.30356 (15) | 0.0128 (5)                       |
| H6   | 0.2160     | 0.5165     | 0.2963       | 0.015*                           |
| C21  | 1.1135 (3) | 0.6280 (4) | 0.86604 (17) | 0.0157 (6)                       |
| H21A | 1.0606     | 0.6433     | 0.9190       | 0.019*                           |
| H21B | 1.2300     | 0.6325     | 0.8877       | 0.019*                           |
| C20  | 0.8278 (3) | 0.3921 (4) | 0.92659 (16) | 0.0146 (6)                       |
| H20A | 0.8416     | 0.5105     | 0.9583       | 0.018*                           |
| H20B | 0.7408     | 0.3256     | 0.9481       | 0.018*                           |
| C19  | 1.0918 (3) | 0.3042 (4) | 0.90154 (16) | 0.0146 (6)                       |
| C18  | 1.0696 (3) | 0.4405 (4) | 0.82395 (14) | 0.0117 (5)                       |
| C17  | 0.6166 (3) | 0.4422 (4) | 0.78970 (15) | 0.0105 (5)                       |
| C16  | 0.7825 (3) | 0.4245 (4) | 0.82765 (15) | 0.0117 (5)                       |
| C15  | 0.8984 (3) | 0.4369 (4) | 0.77579 (15) | 0.0102 (5)                       |
| C14  | 0.8595 (3) | 0.4540 (4) | 0.68131 (14) | 0.0111 (5)                       |
| H14  | 0.9396     | 0.4564     | 0.6448       | 0.013*                           |
| C13  | 0.7028 (3) | 0.4669 (4) | 0.64496 (14) | 0.0106 (5)                       |
| C12  | 0.4254 (3) | 0.4900 (4) | 0.64572 (14) | 0.0115 (5)                       |
| H12A | 0.3755     | 0.6036     | 0.6625       | 0.014*                           |
| H12B | 0.3564     | 0.3854     | 0.6541       | 0.014*                           |
| C11  | 0.6212 (3) | 0.4838 (4) | 0.55175 (14) | 0.0098 (5)                       |

|      |            |            |              |            |
|------|------------|------------|--------------|------------|
| C10  | 0.4570 (3) | 0.4985 (4) | 0.55105 (15) | 0.0108 (5) |
| C7   | 0.3531 (3) | 0.5150 (4) | 0.47127 (15) | 0.0100 (5) |
| C8   | 0.1777 (3) | 0.5392 (4) | 0.46971 (16) | 0.0128 (5) |
| H8A  | 0.1328     | 0.6163     | 0.4175       | 0.015*     |
| H8B  | 0.1623     | 0.6039     | 0.5251       | 0.015*     |
| C22  | 1.0666 (4) | 0.7842 (5) | 0.8020 (2)   | 0.0258 (7) |
| H22A | 0.9502     | 0.7935     | 0.7882       | 0.031*     |
| H22B | 1.1080     | 0.7631     | 0.7462       | 0.031*     |
| H22C | 1.1112     | 0.8976     | 0.8300       | 0.031*     |
| C9   | 0.0871 (3) | 0.3616 (5) | 0.4636 (2)   | 0.0206 (6) |
| H9A  | -0.0263    | 0.3862     | 0.4619       | 0.025*     |
| H9B  | 0.1280     | 0.2863     | 0.5162       | 0.025*     |
| H9C  | 0.1010     | 0.2971     | 0.4086       | 0.025*     |

*Atomic displacement parameters (Å<sup>2</sup>)*

|     | $U^{11}$    | $U^{22}$    | $U^{33}$    | $U^{12}$     | $U^{13}$    | $U^{23}$     |
|-----|-------------|-------------|-------------|--------------|-------------|--------------|
| O1  | 0.0172 (8)  | 0.0388 (14) | 0.0013 (7)  | 0.0003 (10)  | 0.0016 (6)  | 0.0001 (8)   |
| N1  | 0.0113 (8)  | 0.0186 (13) | 0.0034 (8)  | -0.0004 (9)  | 0.0030 (7)  | 0.0013 (8)   |
| C1  | 0.0161 (11) | 0.0200 (15) | 0.0045 (10) | -0.0002 (11) | 0.0010 (8)  | 0.0010 (10)  |
| O2  | 0.0136 (8)  | 0.0293 (12) | 0.0031 (7)  | -0.0004 (8)  | 0.0059 (6)  | -0.0008 (7)  |
| N2  | 0.0091 (8)  | 0.0187 (13) | 0.0032 (8)  | 0.0007 (9)   | 0.0031 (6)  | -0.0004 (8)  |
| C2  | 0.0176 (11) | 0.0268 (17) | 0.0048 (9)  | 0.0008 (12)  | 0.0061 (8)  | 0.0002 (10)  |
| C3  | 0.0118 (10) | 0.0246 (16) | 0.0062 (9)  | 0.0001 (11)  | 0.0048 (8)  | 0.0016 (10)  |
| O3  | 0.0151 (8)  | 0.0331 (13) | 0.0062 (7)  | 0.0032 (8)   | 0.0047 (6)  | 0.0080 (8)   |
| C4  | 0.0124 (10) | 0.0173 (15) | 0.0035 (9)  | -0.0005 (11) | 0.0028 (8)  | 0.0008 (9)   |
| O4  | 0.0191 (10) | 0.0324 (14) | 0.0143 (9)  | 0.0067 (9)   | 0.0052 (7)  | 0.0084 (8)   |
| C5  | 0.0112 (10) | 0.0149 (14) | 0.0042 (9)  | 0.0000 (10)  | 0.0028 (7)  | 0.0011 (9)   |
| O5  | 0.0085 (8)  | 0.0340 (14) | 0.0039 (7)  | 0.0037 (8)   | 0.0048 (6)  | -0.0018 (7)  |
| OW1 | 0.0227 (10) | 0.0524 (17) | 0.0046 (8)  | -0.0111 (10) | 0.0075 (7)  | -0.0076 (9)  |
| C6  | 0.0117 (10) | 0.0206 (15) | 0.0058 (10) | -0.0007 (11) | 0.0010 (8)  | 0.0001 (10)  |
| C21 | 0.0153 (12) | 0.0241 (16) | 0.0080 (10) | -0.0014 (11) | 0.0028 (9)  | -0.0026 (10) |
| C20 | 0.0133 (11) | 0.0270 (18) | 0.0045 (10) | 0.0018 (11)  | 0.0039 (8)  | 0.0009 (10)  |
| C19 | 0.0152 (12) | 0.0233 (16) | 0.0055 (10) | -0.0002 (11) | 0.0023 (9)  | 0.0001 (10)  |
| C18 | 0.0102 (10) | 0.0237 (15) | 0.0024 (9)  | 0.0009 (10)  | 0.0044 (7)  | 0.0003 (9)   |
| C17 | 0.0124 (10) | 0.0166 (14) | 0.0035 (9)  | -0.0004 (10) | 0.0042 (8)  | -0.0007 (9)  |
| C16 | 0.0126 (10) | 0.0202 (15) | 0.0029 (9)  | 0.0000 (10)  | 0.0034 (8)  | 0.0005 (9)   |
| C15 | 0.0116 (10) | 0.0146 (14) | 0.0047 (9)  | 0.0010 (10)  | 0.0026 (8)  | 0.0011 (9)   |
| C14 | 0.0123 (10) | 0.0185 (15) | 0.0037 (9)  | -0.0005 (10) | 0.0046 (8)  | -0.0001 (9)  |
| C13 | 0.0127 (10) | 0.0169 (15) | 0.0035 (9)  | -0.0009 (10) | 0.0048 (7)  | 0.0004 (9)   |
| C12 | 0.0097 (9)  | 0.0230 (16) | 0.0024 (9)  | 0.0014 (11)  | 0.0030 (7)  | -0.0004 (10) |
| C11 | 0.0099 (9)  | 0.0157 (14) | 0.0040 (9)  | 0.0003 (10)  | 0.0021 (7)  | 0.0008 (9)   |
| C10 | 0.0129 (10) | 0.0160 (14) | 0.0046 (9)  | -0.0011 (11) | 0.0045 (8)  | 0.0001 (9)   |
| C7  | 0.0112 (10) | 0.0138 (14) | 0.0059 (10) | -0.0010 (10) | 0.0040 (8)  | 0.0006 (9)   |
| C8  | 0.0111 (11) | 0.0203 (16) | 0.0073 (10) | 0.0029 (10)  | 0.0029 (8)  | -0.0002 (9)  |
| C22 | 0.0238 (14) | 0.0229 (18) | 0.0296 (16) | -0.0054 (13) | 0.0013 (12) | 0.0025 (13)  |
| C9  | 0.0123 (12) | 0.0255 (18) | 0.0243 (14) | -0.0011 (11) | 0.0048 (10) | 0.0016 (12)  |

Geometric parameters (Å, °)

|            |             |              |             |
|------------|-------------|--------------|-------------|
| O1—C1      | 1.354 (3)   | C21—H21B     | 0.9900      |
| O1—H1      | 0.8400      | C20—C16      | 1.492 (3)   |
| N1—C11     | 1.315 (3)   | C20—H20A     | 0.9900      |
| N1—C4      | 1.364 (3)   | C20—H20B     | 0.9900      |
| C1—C6      | 1.378 (3)   | C19—C18      | 1.526 (4)   |
| C1—C2      | 1.408 (3)   | C18—C15      | 1.511 (3)   |
| O2—C17     | 1.248 (3)   | C17—C16      | 1.435 (3)   |
| N2—C13     | 1.372 (3)   | C16—C15      | 1.368 (3)   |
| N2—C17     | 1.374 (3)   | C15—C14      | 1.410 (3)   |
| N2—C12     | 1.475 (3)   | C14—C13      | 1.356 (3)   |
| C2—C3      | 1.363 (3)   | C14—H14      | 0.9500      |
| C2—H2      | 0.9500      | C13—C11      | 1.459 (3)   |
| C3—C4      | 1.415 (3)   | C12—C10      | 1.500 (3)   |
| C3—H3      | 0.9500      | C12—H12A     | 0.9900      |
| O3—C19     | 1.327 (3)   | C12—H12B     | 0.9900      |
| O3—C20     | 1.460 (3)   | C11—C10      | 1.403 (3)   |
| C4—C5      | 1.428 (3)   | C10—C7       | 1.367 (3)   |
| O4—C19     | 1.200 (3)   | C7—C8        | 1.502 (3)   |
| C5—C6      | 1.413 (3)   | C8—C9        | 1.511 (4)   |
| C5—C7      | 1.432 (3)   | C8—H8A       | 0.9900      |
| O5—C18     | 1.409 (3)   | C8—H8B       | 0.9900      |
| O5—H5      | 0.8400      | C22—H22A     | 0.9800      |
| OW1—HW1A   | 0.839 (13)  | C22—H22B     | 0.9800      |
| OW1—HW1B   | 0.836 (13)  | C22—H22C     | 0.9800      |
| C6—H6      | 0.9500      | C9—H9A       | 0.9800      |
| C21—C22    | 1.508 (4)   | C9—H9B       | 0.9800      |
| C21—C18    | 1.536 (4)   | C9—H9C       | 0.9800      |
| C21—H21A   | 0.9900      |              |             |
|            |             |              |             |
| C1—O1—H1   | 109.5       | O2—C17—C16   | 125.9 (2)   |
| C11—N1—C4  | 114.48 (18) | N2—C17—C16   | 113.8 (2)   |
| O1—C1—C6   | 118.6 (2)   | C15—C16—C17  | 121.8 (2)   |
| O1—C1—C2   | 121.0 (2)   | C15—C16—C20  | 119.7 (2)   |
| C6—C1—C2   | 120.3 (2)   | C17—C16—C20  | 118.4 (2)   |
| C13—N2—C17 | 124.44 (19) | C16—C15—C14  | 121.2 (2)   |
| C13—N2—C12 | 112.99 (18) | C16—C15—C18  | 117.46 (19) |
| C17—N2—C12 | 122.53 (19) | C14—C15—C18  | 121.2 (2)   |
| C3—C2—C1   | 120.1 (2)   | C13—C14—C15  | 117.0 (2)   |
| C3—C2—H2   | 119.9       | C13—C14—H14  | 121.5       |
| C1—C2—H2   | 119.9       | C15—C14—H14  | 121.5       |
| C2—C3—C4   | 121.2 (2)   | C14—C13—N2   | 121.5 (2)   |
| C2—C3—H3   | 119.4       | C14—C13—C11  | 131.6 (2)   |
| C4—C3—H3   | 119.4       | N2—C13—C11   | 106.93 (18) |
| C19—O3—C20 | 121.8 (2)   | N2—C12—C10   | 101.73 (17) |
| N1—C4—C3   | 117.1 (2)   | N2—C12—H12A  | 111.4       |
| N1—C4—C5   | 124.07 (19) | C10—C12—H12A | 111.4       |

|               |             |               |             |
|---------------|-------------|---------------|-------------|
| C3—C4—C5      | 118.9 (2)   | N2—C12—H12B   | 111.4       |
| C6—C5—C4      | 118.62 (19) | C10—C12—H12B  | 111.4       |
| C6—C5—C7      | 123.0 (2)   | H12A—C12—H12B | 109.3       |
| C4—C5—C7      | 118.36 (19) | N1—C11—C10    | 126.5 (2)   |
| C18—O5—H5     | 109.5       | N1—C11—C13    | 124.9 (2)   |
| HW1A—OW1—HW1B | 107 (2)     | C10—C11—C13   | 108.63 (19) |
| C1—C6—C5      | 120.7 (2)   | C7—C10—C11    | 120.3 (2)   |
| C1—C6—H6      | 119.6       | C7—C10—C12    | 130.0 (2)   |
| C5—C6—H6      | 119.6       | C11—C10—C12   | 109.68 (19) |
| C22—C21—C18   | 113.7 (2)   | C10—C7—C5     | 116.2 (2)   |
| C22—C21—H21A  | 108.8       | C10—C7—C8     | 120.8 (2)   |
| C18—C21—H21A  | 108.8       | C5—C7—C8      | 122.9 (2)   |
| C22—C21—H21B  | 108.8       | C7—C8—C9      | 113.3 (2)   |
| C18—C21—H21B  | 108.8       | C7—C8—H8A     | 108.9       |
| H21A—C21—H21B | 107.7       | C9—C8—H8A     | 108.9       |
| O3—C20—C16    | 111.8 (2)   | C7—C8—H8B     | 108.9       |
| O3—C20—H20A   | 109.3       | C9—C8—H8B     | 108.9       |
| C16—C20—H20A  | 109.3       | H8A—C8—H8B    | 107.7       |
| O3—C20—H20B   | 109.3       | C21—C22—H22A  | 109.5       |
| C16—C20—H20B  | 109.3       | C21—C22—H22B  | 109.5       |
| H20A—C20—H20B | 107.9       | H22A—C22—H22B | 109.5       |
| O4—C19—O3     | 119.8 (2)   | C21—C22—H22C  | 109.5       |
| O4—C19—C18    | 122.3 (2)   | H22A—C22—H22C | 109.5       |
| O3—C19—C18    | 117.8 (2)   | H22B—C22—H22C | 109.5       |
| O5—C18—C15    | 108.33 (18) | C8—C9—H9A     | 109.5       |
| O5—C18—C19    | 109.7 (2)   | C8—C9—H9B     | 109.5       |
| C15—C18—C19   | 109.6 (2)   | H9A—C9—H9B    | 109.5       |
| O5—C18—C21    | 111.7 (2)   | C8—C9—H9C     | 109.5       |
| C15—C18—C21   | 111.0 (2)   | H9A—C9—H9C    | 109.5       |
| C19—C18—C21   | 106.5 (2)   | H9B—C9—H9C    | 109.5       |
| O2—C17—N2     | 120.3 (2)   |               |             |

Hydrogen-bond geometry ( $\text{\AA}$ ,  $^\circ$ )

| $D-H\cdots A$                       | $D-H$    | $H\cdots A$ | $D\cdots A$ | $D-H\cdots A$ |
|-------------------------------------|----------|-------------|-------------|---------------|
| O1—H1 $\cdots$ OW1                  | 0.84     | 1.91        | 2.734 (3)   | 168           |
| O5—H5 $\cdots$ O2 <sup>i</sup>      | 0.84     | 2.06        | 2.894 (3)   | 172           |
| OW1—HW1A $\cdots$ O2 <sup>ii</sup>  | 0.84 (1) | 2.05 (2)    | 2.879 (3)   | 170 (4)       |
| OW1—HW1B $\cdots$ O4 <sup>iii</sup> | 0.84 (1) | 2.09 (2)    | 2.894 (3)   | 162 (4)       |

Symmetry codes: (i)  $x+1, y, z$ ; (ii)  $x, y, z-1$ ; (iii)  $-x+2, y+1/2, -z+1$ .