Single-crystal-to-single-crystal phase transition of 18β-glycyrrhetic acid isopropyl ester

Dominik Langer, Barbara Wicher* and Ewa Tykarska*

Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Grunwaldzka 6, Poznan 60-780, Poland. *Correspondence e-mail: bwicher@ump.edu.pl, etykarsk@ump.edu.pl

Due to the destruction of the integrity of the parent crystal, single-crystal-to-single-crystal phase transition in organic compounds is still a relatively rare phenomenon. The phase transition in glycyrrhetic acid isopropyl ester is triggered by temperature change. The increasing volume of the isopropyl substituent as a result of increasing temperature forces a remodelling of the structural motifs. These changes cause a single-crystal-to-single-crystal phase transition. The low-temperature form is isostructural with glycyrrhetic acid methanol solvate, while the high-temperature phase is isostructural with the ethyl ester of this acid.

1. Introduction

Reports of using liquorice as a remedy for the treatment of gastrointestinal or respiratory ailments are as old as the literature related to medicine. It was already ‘prescribed’ in ancient Egypt, China, India, Greece and Rome (Fiore et al., 2005, 2008). Triterpene saponins, in particular glycyrrhizic acid (GA) in the form of different salts, are the main active compounds derived from liquorice. 18β-Glycyrrhetic acid (GE), in turn, is an aglycon of GA (Scheme 1). GE shows broad pharmacological properties, such as anti-inflammatory (Radwan et al., 2016; Quan et al., 2021; Feng et al., 2021), antiviral (Khwaza et al., 2018; Wang et al., 2012) cytostatic or proapoptotic activities (Wang et al., 2019; Feng et al., 2021; Luo et al., 2021). Additionally, derivatives containing lipophilic moieties, e.g. ester groups, exhibit cytotoxicity against hepatic (Hep-G2) (Wang et al., 2018) and human breast (MCF-7) cancer cell lines (Wang et al., 2019).

Glycyrrhetic acid (GE) is composed of an elongated triterpene core with hydroxy and carboxyl groups at opposite
ends, and a carbonyl group in the middle (Scheme 1). The chemical transformation of this acid to the ester is relatively straightforward and is performed using alkyl halides (Schwarz & Csuk, 2010; Langer et al., 2016).

In the course of our analysis of the crystal structures of different triterpenes (Tykarska et al., 2012, 2014; Tykarska & Gdaniec, 2013, 2015), we have synthesized the ethyl, isopropyl and 2-morpholinooethyl GE esters (Langer et al., 2016). By modifying the carboxyl group to lipophilic ester groups, we reduced the ability of esters to form hydrogen bonds, thus causing van der Waals interactions to play a vital role in the crystal packing of GE esters.

The crystal structures of all the synthesized esters (Scheme 1) were determined at 130 K in the orthorhombic space group $P_{2_1}2_12_1$ (Langer et al., 2016). Surprisingly, despite pronounced differences in the volume and the rigidity of the ester substituents, their structures were similar, though not identical. The repeating motif formed by hydrogen bonds is a chain of molecules extended along [010]. However, an analysis of the crystal packing from the point of view of van der Waals interactions revealed another recurring one-dimensional (1D) motif, namely, a zigzag ribbon extended along [100]. Ribbons aggregate into (001) layers that stack along the [001] direction, forming layered three-dimensional (3D) structures (Fig. 1). Hydrogen bonds link molecules from adjacent (001) layers. It should be stressed that these structures cannot be referred to as isostructural despite a similar scheme of molecular association. The differences in the relative orientations of the molecules in the structural motifs result in noticeable dissimilarities in the overall crystal packing. It is also worth mentioning that the structure of GE methanol solvate (Alvarez-Larena et al., 2007) resembles the crystal architecture of the ester since methanol molecules occupy the positions of the ester substituents in the crystals (Langer et al., 2016).

Our subsequent research focusing on the cocrystallization of GE esters gave an unexpected result and revealed a single-crystal-to-single-crystal phase transition occurring in isopropyl-GE ester. It could be captured by differential scanning calorimetry (DSC) and single-crystal X-ray diffraction (SXRD) experiments. In the course of this article, we will discuss differences in the architectures of the structural motifs of two phases of isopropyl-GE ester and show the structural relationship of both phases to the ethyl and 2-morpholinooethyl esters and GE methanol solvate.

Phase transitions in organic compounds are not an uncommon phenomenon (Zakharov et al., 2018; Li et al., 2019; Khoj et al., 2013; Palmer et al., 2012; Hirai et al., 2020; Colmenero, 2020; de Moura Oliveira et al., 2021; Rok et al., 2017). However, very often, during phase transformation, profound changes in the crystal structures cause the loss of crystal integrity. Thus, single-crystal-to-single-crystal transitions are still relatively rare (Kariuki & El-Hiti, 2017; Matos et al., 2021; Rubín-Preminger et al., 2004; Pete et al., 2015; Sriramambhatla et al., 2020).

2. Experimental

2.1. Synthesis and crystallization

All reagents were of analytical grade and were purchased from commercial suppliers (Merck, Witko CHS) and used without further purification. Isopropyl-GE ester was synthesized using the previously reported procedure of Langer et al. (2016), which was a slightly modified version of the procedure reported by Schwarz & Csuk (2010).

Single crystals suitable for X-ray diffraction experiments were obtained by dissolving 6 mg of crude isopropyl-GE in a mixture of dichloromethane and acetone (for P-11-18) or in ethyl acetate (for P-13-17).

Single crystals of the ethyl-GE and 2-morpholinooethyl-GE esters were obtained from dimethylformamide (DMF).

2.2. Characterization techniques

2.2.1. Single-crystal X-ray diffraction. Crystal data, data collection and structure refinement details are summarized in Table 1. The absolute structures of the GE esters are supported by Flack parameters (Table 1) and conform with the known absolute configuration of GE. All non-H atoms, apart from those of the disordered methyl groups in ethyl-GE and P-13-17, were refined anisotropically. The C-bound H atoms were constrained to their calculated positions and were refined as riding on their pivot atoms, with $U_{iso}(H) = 1.2U_{eq}(C)$ or $1.5U_{eq}$(methyl C). The H atoms of the O—H groups were located in difference electron-density maps but, for further refinement, O—H distances were standardized to 0.82 Å and $U_{iso}(H)$ values were set at 1.2$U_{eq}$(O). For refinement of the P-13-17 structure, the DFIX and EADP restraints were used to model disordered isopropyl group geometry and atomic displacement parameters.

The diffraction data used for the determination of the unit-cell parameters during phase transition were collected in heating–cooling–heating cycles. The single crystal of phase P-11-18 was first heated from 300 to 340 K, then cooled to 280 K and warmed again to 340 K. The temperature was controlled using a Cryostream Controller 700. The temper-
Table 1: Experimental details.

For all structures: orthorhombic, \( P_{2_1}2_12_1 \), \( Z = 4 \). Experiments were carried out at 298 K with Cu K\( \alpha \) radiation using a Rigaku OD SuperNova Dual source diffractometer with an Atlas detector. Absorption was corrected for by multi-scan methods, (CrysAlis PRO: Rigaku OD, 2015). H-atom parameters were constrained.

<table>
<thead>
<tr>
<th></th>
<th>P_11_18</th>
<th>P_13_17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>C(<em>3)H(</em>{52})O(_4)</td>
<td>C(<em>3)H(</em>{52})O(_4)</td>
</tr>
<tr>
<td>( M_1 )</td>
<td>512.74</td>
<td>512.74</td>
</tr>
<tr>
<td>( a, b, c ) (( \text{\AA} ))</td>
<td>11.8862 (3), 13.1419 (3), 18.8884 (4)</td>
<td>13.1807 (3), 13.2466 (4), 17.2198 (5)</td>
</tr>
<tr>
<td>( V ) (( \text{\AA}^3 ))</td>
<td>2950.51 (12)</td>
<td>3906.57 (13)</td>
</tr>
<tr>
<td>( \mu ) (mm(^{-1}))</td>
<td>0.56</td>
<td>0.56</td>
</tr>
<tr>
<td>Crystal size (mm)</td>
<td>0.43 ( \times ) 0.4 ( \times ) 0.2</td>
<td>0.4 ( \times ) 0.3 ( \times ) 0.2</td>
</tr>
</tbody>
</table>

| Data collection | \( T_{\text{max}} \) \( T_{\text{min}} \) | 0.879, 1.000 | 0.686, 1.000 |
| No. of measured, independent and observed reflections | 5641, 5641, 5087 | 17521, 5936, 5661 |
| \( R_{\text{int}} \) | 0.023 | 0.017 |
| \( \sin \theta / \lambda_{\text{max}} \) (\( \text{\AA}^{-1} \)) | 0.631 | 0.625 |

| Refinement | \( R(F^2 > 2\sigma(F^2)), wR(F^2), S \) | 0.039, 0.114, 1.07 | 0.054, 0.166, 1.05 |
| No. of reflections | 5641 | 5936 |
| No. of parameters | 344 | 355 |
| No. of restraints | 0 | 13 |
| \( \Delta \rho_{\text{max}}, \Delta \rho_{\text{min}} \) (e \( \text{\AA}^{-3} \)) | 0.15, -0.16 | 0.35, -0.31 |
| Absolute structure | Flack \( x \) determined using 1837 quotients | Flack \( x \) determined using 2272 quotients |
| | \( [(I^+ - (I^-))/[(I^+) + (I^-)] \) (Parsons et al., 2013) | \([([I^+] - (I^-))/[(I^+) + (I^-)] \) (Parsons et al., 2013) |
| Absolute structure parameter | -0.11 (13) | 0.01 (7) |

Computer programs: CrysAlis PRO (Rigaku OD, 2015), SHELXT2014 (Sheldrick, 2015a), SHELXL2018 (Sheldrick, 2015b) and OLEX2 (Dolomanov et al., 2009).

The structural voids used for analysis were calculated using Mercury (Macrae et al., 2020), with a probe radius of 1.2 \( \text{\AA} \) and a grid spacing of 0.7 \( \text{\AA} \).

The crystal structures of ethyl-GE, P-11-18 and the 2-moropholinoethyl-GE esters determined at 130 K were reported previously (Langer et al., 2016). In this article, the structures determined at 298 K will be discussed.

2.2.2. Differential scanning calorimetry (DSC). A DSC 214 Nevio (NETZSCH, Selb, Germany) instrument was utilized to collect the DSC signal. Samples were enclosed in aluminium pans with a small hole in the cover. The measurements were performed under a nitrogen atmosphere (flow rate of 30 ml min\(^{-1}\)).

The pure sample of P-11-18 used for DSC measurements was obtained by freezing the isopropyl-GE ester powder to 253.1 K for 12 h. The DSC program was straightforward. First, the P-11-18 sample was heated to 353.1 K and cooled to 243.1 K, and then heated to 353.1 K and cooled to 253.1 K, and finally heated to 523.1 K. The heating rate was constant, \( i.e. \) 5 K min\(^{-1}\).

2.3. Thermogravimetric analysis (TGA)

TGA was performed using a TG 209 F3 Tarsus instrument (NETZSCH, Selb, Germany). An open corundum crucible was used for measurements. The analyzed samples were heated at a rate of 5 K min\(^{-1}\) from 308.15 to 623.15 K under a nitrogen atmosphere (flow rate of 30 ml min\(^{-1}\)).

2.4. XPac calculations

Similarities of the crystal structures were quantified using the XPac (Gelbrich & Hursthouse, 2005) program. The dissimilarity indices were calculated in the manner described by Gelbrich & Hursthouse (2005) and Gelbrich et al. (2012). For comparison, 22 C atoms of the triterpene core were taken into account. During calculations, the cut-off parameters \( a/p/d \) (angles/planes/distances) were set at 10/14/1.5 \( \text{\AA} \), respectively.

3. Results and discussion

The first indication of the unusual behaviour of isopropyl-GE ester was noticed during attempts to collect diffraction data for the first batch of crystals. The crystals obtained directly from the mother liquor were placed at 130 K in a nitrogen gas stream. We were unable to collect high-quality diffraction data due to the images indicating, in the best case, twinning of the crystals, but generally showing the diffraction pattern of multicrystals. This was surprising because an adhesion of crystals was not detected upon visual inspection under a polarizing microscope. Nevertheless, at this point, we decided to repeat the crystallization to obtain better-quality single crystals. The measurement procedure for the second batch was...
similar, but this time we collected data that were later published in the article concerning the crystal structures of GE esters (Langer et al., 2016). However, our next project dealing with the synthesis of cocrystals of GE esters gave unexpected results. When screening batches of crystals obtained for cocrystallization experiments at room temperature, we assigned two sets of orthorhombic unit-cell parameters for isopropyl-GE ester. The first set was nearly identical to that already published (Langer et al., 2016), with differences arising from the different temperatures, i.e. 11.7789 (2), 13.0812 (3) and 18.7845 (4) Å at 130 K versus 11.8862 (3), 13.1419 (3) and 18.8884 (4) Å at room temperature. The second set of parameters, i.e. 13.1807 (3), 13.2466 (4) and 17.2198 (5) Å, is to some extent related, but the differences could not be caused by the different temperatures. A comparison of the unit-cell parameters shows an unusual change in the lengths of \(a\) and \(c\), while \(b\) is unaffected. The diffraction data for both sets were measured at room temperature, the crystal structures refined and in the course of this article will be denoted as P-11-18 and P-13-17, respectively (Table 1).

At first glance, a comparison of the two structures revealed only minor changes. Both phases have one molecule of isopropyl-GE ester in the asymmetric unit (Figs. 2 and 3). The organization scheme is the same; the molecules self-assemble into zigzag ribbons then into \((001)\) layers from which a layered 3D structure is formed. Adjacent layers are connected via \(O3—H3/C1/C1/C1/O11\) hydrogen bonds. The most obvious difference is seen for the isopropyl substituent, which in P-13-17 shows a large thermal motion and was modelled as disordered over two positions. The occupancy factor for the major part is 0.675 (6). However, the differences between these two positions are not significant. The torsion angle \(C19—C20—C30—O31\) in P-11-18 is 162.2 (3)°, while in P-13-17 for the major position the angle is 146.4 (6)°, and for the minor position is 159.4 (14)° (Fig. 3). Thus, this slight change could not cause a noticeable phase transition. A detailed analysis showed that although the disorder of the isopropyl group is not significant, it substantially impacts the crystal packing. Structural transformation is needed in P-13-17 to accommodate the larger volume occupied with the ester substituent in the crystal.

3.1. Comparison of 1D motifs

As mentioned above, the scheme of molecule association into zigzag ribbons and hydrogen-bonded chains is unchanged, but the construction of these 1D motifs differs in detail.

3.1.1. Zigzag ribbon. The zigzag ribbons can be compared using parameter \(R_s\), introduced to describe the orientation of molecules in the ribbons (Tykarska & Gdaniec, 2015). \(R_s\) is expressed as the dihedral angle between two planes. The first plane, i.e. the ribbon plane, is calculated through 44 C atoms of the triterpene core of two ester molecules related by translation along \([100]\), and the second through atoms \(C9, C11—C14\) and \(O11\) (Table 2). Since the zigzag ribbon is composed of two antiparallel-oriented rows of molecules, the ribbon planes are calculated for half of the ribbon, i.e. a single row (Fig. 4).

<table>
<thead>
<tr>
<th>(R)</th>
<th>(S)</th>
<th>(\Delta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-11-18</td>
<td>10.1</td>
<td>29.3</td>
</tr>
<tr>
<td>P-13-17</td>
<td>39.0</td>
<td>33.0</td>
</tr>
<tr>
<td>Ethyl-GE</td>
<td>41.2</td>
<td>30.2</td>
</tr>
<tr>
<td>2-Morpholinoethyl-GE</td>
<td>24.0</td>
<td>45.7</td>
</tr>
<tr>
<td>Methanol-GE*</td>
<td>10.7</td>
<td>21.3</td>
</tr>
</tbody>
</table>

Note: (*) calculated based on the crystal structure from Alvarez-Larena et al. (2007).

Figure 2
The asymmetric unit and atom-labelling scheme (C atoms have the prefix ’C’ omitted) for P-11-18. Displacement ellipsoids are drawn at the 50% probability level. The H atoms have been omitted for clarity.

Figure 3
The orientation of the isopropyl substituent in P-11-18 and P-13-17. The minor position of the disordered isopropyl group in the P-13-17 phase is coloured green. The H atoms have been omitted for clarity.

Table 2
\(R\), \(S\) (°) and \(\Delta\) (Å) parameters at room temperature.
\(R\) is rotation of molecules within the ribbon, \(S\) is the slope of the ribbon to the layer surface – \((001)\) plane, \(\Delta\) characterizes the width of the layer.

Figure 3
The orientation of the isopropyl substituent in P-11-18 and P-13-17. The minor position of the disordered isopropyl group in the P-13-17 phase is coloured green. The H atoms have been omitted for clarity.
130 K (Langer et al., 2016) and room temperature (Table S2 in the supporting information) for 2-morpholinoethyl-GE ester is typical of the expected thermal expansion, and no change in the crystal packing is observed. Slight distortions are observed for ethyl-GE ester, i.e. 12.9577 (5), 13.0369 (4) and 16.3908 (5) Å at 130 K (Langer et al., 2016) versus 13.0243 (1), 12.9924 (1) and 16.6941 (2) Å at room temperature. However, this unexpected change in the unit-cell parameters does not influence the crystal packing or the parameters describing the structural motifs. The ethyl substituent was modelled as disordered at room temperature. The structure of GE methanol solvate was established at room temperature by Alvarez-Larena et al. (2007).

The difference of 28.9° in the R values (Table 2) indicates a pronounced alteration between the ribbons formed in the P-13-17 and P-11-18 phases. Changes in the rotation of the molecules are reflected in the unit-cell parameters a and c. The elongation of the a parameter in the P-13-17 phase results from a shift of the ester molecules along [100]. The shortening of the c parameter is correlated with the displacement of adjacent layers and is discussed below in §3.3.

The R values in Table 2 indicate the similarity of the P-13-17 phase to the ethyl-GE ester and the P-11-18 phase to the GE methanol solvate structure. Astonishingly, the value of the rotation index R for 2-morpholinoethyl-GE ester is a geometrical mean of the R values of the P-13-17 and P-11-18 phases.

Table 3

<table>
<thead>
<tr>
<th></th>
<th>D−H⋯A</th>
<th>D−H⋯A</th>
<th>D−H⋯A</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-11-18</td>
<td>O3⋯H3A···O11' 0.82 2.05 2.849 (3) 166</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-13-17</td>
<td>O3⋯H3···O11' 0.82 2.24 3.044 (4) 169</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl-GE</td>
<td>O3⋯H3···O11' 0.82 2.28 3.081 (3) 166</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Morpholinoethyl-GE</td>
<td>O3⋯H3···O11' 0.82 2.00 2.808 (2) 169</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol-GE</td>
<td>O3⋯H3···O11' 0.82 2.13 2.926 (4) 165</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: (*) calculated based on the crystal structure from Alvarez-Larena et al. (2007). Symmetry codes: (i) −x + 1, y − 1/2, −z + 1/2; (ii) −x, −y, −z.

3.1.2. Hydrogen-bonded chain. Another 1D motif found in the GE esters is a chain consisting of molecules that are joined by directional O3⋯H3···O11 interactions. In the studied structures, this chain is formed at the interface of layers in the [010] direction.

A comparison of the hydrogen-bond parameters showed a significant elongation of the H⋯A and D⋯A distances in P-13-17 compared to P-11-18 (Table 3). These changes are a consequence of the rebuilding of the ribbons. Rotation of the molecules within the ribbons leads to different positions of the O11 carbonyl groups on the surface of the layers, thus changing the hydrogen-bond geometry.

Additionally, a comparison of the hydrogen-bond parameters of both isopropyl-GE phases with other esters and GE methanol solvate shows that P-13-17 resembles ethyl-GE ester, while P-11-18 is similar to 2-morpholinoethyl-GE ester and the solvate.

3.2. Comparison of (001) layers

The other two parameters presented in Table 2 were introduced to provide a description of the layers in triterpene structures (Tykarska & Gdaniec, 2015). The values of factors S and Δ characterize the slope of the ribbons with respect to the layer surface and the layer width, respectively. S is defined as the dihedral angle between the ribbon plane, calculated as given for R, and the plane representing the (001) layer surface calculated through the C3 atoms of molecules related by translation along the two shortest unit-cell parameters. In turn, Δ is the distance between two (001) planes calculated through the C3 atoms of molecules arranged in an antiparallel manner within the layer.

The differences in the S values for all compared structures are not as significant as for R. However, the elevated values for 2-morpholinoethyl-GE ester distinguish this structure from the others. The difference in the S value between P-11-18 and the methanol solvate of GE is 8°, while for the pair
P-13-17 and ethyl-GE ester the difference is only \( \frac{2}{\sqrt{14}} \), pointing to minor dissimilarities between the latter pair. Similarly, all esters except 2-morpholinoethyl-GE have a comparable \( \Delta \) value, indicating an almost identical layer width (Fig. 5).

3.3. Comparison of the crystal packing

As mentioned above, rotations of the molecules within the ribbon cause differences in the unit-cell parameters \( a \) and \( c \). But these also influence the interlayer contacts and, as a result, the crystal packing.

The shorter \( a \) parameter of the unit cell in P-11-18 means a closer packing of molecules in the layer along the [100] direction compared to P-13-17. This denser structure causes adjacent two-dimensional (2D) units to be separated and not overlapped [Fig. 6(a)]. In turn, the longer parameter \( a \) in P-13-17 means a less dense packing of the molecules. Due to the increased distance between the molecules in the ribbon, the construction of the surfaces of the layers changes, which allows for a slight interpenetration of the triterpenes of adjacent 2D units and, as a consequence, a shortening of parameter \( c \) [Fig. 6(b)]. The mutual displacement of neighbouring layers influences the geometry of the hydrogen bonds and their orientation with respect to the crystallographic axes (Table 3 and Fig. 6).

3.4. Degree of similarity – XPac calculation

From the above comparison of various parameters (Tables 2 and 3), it can be concluded that the P-13-17 phase is similar to ethyl-GE ester, while the P-11-18 phase resembles GE methanol solvate. To measure the degree of similarity, we have used the XPac program. Detailed information about the calculation procedures can be found in Gelbrich & Hursthouse (2005) and Gelbrich et al. (2012). The idea of the calculation is straightforward and based on geometric parameters. A central molecule is paired with the molecules from its closest surroundings; usually, it is 14 neighbours (\( n = 14 \)). The geometry of each pair is described with angular, planar and distance parameters. When comparing two structures, these factors are related, and two parameters, \( x \) and \( d \), are calculated. Index \( x \) reflects the orientational disagreements and is estimated using angles and planes, while \( d \) characterizes the overall changes in the intermolecular distances of two compared structures. For a perfect match, \( x \) and \( d \) are 0.

The XPac calculations confirm the conclusions drawn from the analysis of the hydrogen-bond geometry and the \( R \), \( S \) and \( \Delta \) parameters (Table 4). The phases of isopropyl-GE ester are structurally distinct. The P-13-17 phase is isostructural with ethyl-GE ester, while P-11-18 is isostructural with GE methanol solvate. It is worth pointing out that the \( x \) and \( d \) indices have elevated values for the latter pair. This is consistent with the fact that the differences in the \( S \) and \( \Delta \) parameters for P-11-18 and GE methanol solvate are greater than those estimated for P-13-17 and ethyl-GE ester.
**Table 4**

**XPac** parameters calculated for 22 C atoms of the triterpene core.

Filter settings a/p/d = 10/14/1.5 Å, NS = no similarity and n = number of neighbours. For methanol-GE calculations were performed based on the crystal structure from Alvarez-Larena et al. (2007).

<table>
<thead>
<tr>
<th>Ethyl-GE</th>
<th>P-11-18</th>
<th>P-13-17</th>
<th>2-Morpholinoethyl-GE</th>
<th>Methanol-GE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl-GE –</td>
<td>NS</td>
<td>d = 0.26</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P-11-18 NS</td>
<td>–</td>
<td>NS</td>
<td>d = 0.12</td>
<td>d = 0.23</td>
</tr>
<tr>
<td>P-13-17 x = 4.5, n = 14</td>
<td>NS</td>
<td>x = 13.1, n = 2</td>
<td>–</td>
<td>NS</td>
</tr>
<tr>
<td>2-Morpholinoethyl-GE</td>
<td>NS</td>
<td>x = 13.6, n = 2</td>
<td>–</td>
<td>NS</td>
</tr>
<tr>
<td>Methanol-GE NS</td>
<td>x = 9.1, n = 12</td>
<td>NS</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

**XPac** calculations also confirm that 2-morpholinoethyl-GE ester is not isostructural with any of the other structures. But, as suspected from the $R$ parameter, the zigzag ribbon formed in this structure relates to these observed in P-13-17 and P-11-18. Hence, to some extent, this ribbon can be considered as an intermediate form of the 1D motifs observed in P-13-17 and P-11-18.

3.5. Differential scanning calorimetry and variable-temperature SXRD

To determine the thermodynamic relationship of the P-13-17 and P-11-18 phases, differential scanning calorimetry (DSC) measurements were performed. Prior to this, a thermogravimetric analysis (TGA) experiment was conducted to establish the thermal stability of isopropyl-GE ester. As can be seen in Fig. S1 in the supporting information, the compound is stable up to its melting point.

We knew from the preliminary DSC experiments that the P-11-18 phase is stable at low temperatures, so the DSC scans were collected in the heating–cooling–heating–cooling–heating cycles described in §2.2.2 in the Experimental.

The endothermic peaks characterizing the transition of P-11-18 to P-13-17 are at 317.4 ($T_{\text{onset}} = 311.8$ K), 319.7 ($T_{\text{onset}} = 313.0$ K) and 321.7 K ($T_{\text{onset}} = 308.6$ K) for the first, second and third heating cycles, respectively [Fig. 7(a)]. The exothermic peaks of the reverse transformation have maxima at 284.1 ($T_{\text{onset}} = 279.5$ K) and 287.9 K ($T_{\text{onset}} = 277.0$ K) for the first and second cooling cycles. The determined melting point of isopropyl-GE ester is 487.2 K ($T_{\text{onset}} = 484.6$ K) [Fig. 7(a)]. The shift of the peaks depending on the cycle number is expected behaviour. The temperatures of the thermal events may change along with the thermal history of the sample. This experiment proves that the two phases have an enantiotropic relationship (Burger & Ramberger, 1979). They co-exist in a range of about 35 K.

X-ray diffraction experiments of a single crystal of P-11-18 at variable temperatures were conducted using the heating–cooling–heating–heating program (see §2.2.1 in the Experimental). The unit-cell dimensions were established every 5 K. During both warming cycles, phase P-11-18 transforms to P-13-17 between 325 and 330 K. The reverse change was captured at 300 K (Table S1 in the supporting information). Both unit cells can be determined based on SXRD data. In Fig. 7(b), plots of $a$, $b$, $c$ and $V$ versus temperature are shown. The hysteresis characteristic for a first-order phase transition is observed for $a$ and $c$, and as a consequence for $V$, while $b$ seems unaffected during the phase transition. The X-ray diffraction experiments show that the range of co-existence of the phases is narrower than in the DSC experiment and is about 25 K.

TGA and DSC measurements were also performed for the ethyl-GE and 2-morpholinoethyl-GE esters. As shown in Fig. S3 (see supporting information), both compounds are stable above 600 K. Only one endothermic event is present in the DSC thermograms. The melting point of ethyl-GE is 532.9 K ($T_{\text{onset}} = 530.2$ K) and of 2-morpholinoethyl-GE is 475.4 K ($T_{\text{onset}} = 473.4$ K).

![Figure 7](image-url)
3.6. Structural justification of the isopropyl-GE ester phase transition

The question is: why does the phase transition occur in isopropyl-GE ester while it is not observed in the ethyl- and 2-morpholinoethyl-GE esters?

Along with increasing temperature, the thermal motion of atoms also grows, which is usually expressed in a gradual elongation of the unit-cell parameters. However, in specific cases, a phase transition has to occur to handle changes triggered by rising temperature. In the case of the GE esters, thermal expansion is not uniform throughout the molecule, and the flexible substituents vibrate more than the skeleton atoms. With increasing temperature, the triterpene molecules have to shift and/or rotate to create a bigger volume for the agitated ester substituents.

Somewhat approximated, the presence of empty volumes in the crystal will indicate that the structure is not tightly packed and can cope to some extent with the increasing volume of the ester substituent. In turn, the lack of voids demonstrates very efficient crystal packing and limited accommodation capacity.

In the crystal of 2-morpholinoethyl-GE ester at 130 K (Langer et al., 2016) and at room temperature, voids are observed in the substituent area [Fig. 8(c)]. Free unit-cell volumes are 0.6 (20.1 Å³) and 1.2% (40.9 Å³) at 130 K and room temperature, respectively. Interestingly, in the crystal of the GE ethyl ester at 130 K, no free spaces can be found, but voids with a volume of 2.1% (63.0 Å³) are created at room temperature [Fig. 8(a)]. In both crystals, the temperature-related changes in the volume of the ester substituents did not affect the association of the molecules.

However, there is a temperature-induced phase transition in the GE isopropyl ester crystal. There are no voids at 130 K or at room temperature in the P-11-18 phase, which means that the structure is always tightly packed. But, at some point, the adjusting ability of the crystal for substituent thermal expansion reaches a limit, and the structure transforms to the other phase, i.e. P-13-17. This phase has voids occupying 2.1% (63.9 Å³) of the unit-cell volume. The changes in the structural motifs are significant enough to be noticeable by DSC and X-ray diffraction techniques, but they are gentle enough not to devastate the integrity of the single crystal.

Why during cooling does the phase transition occur in isopropyl-GE but not in ethyl-GE? It seems likely that the type of crystal packing observed in the latter is well suited for the ethyl substituent at higher as well as lower temperatures. In contrast, the P-13-17 structure is suitable only for the strongly vibrating isopropyl group, while efficient packing at lower temperatures can be accomplished only in the P-11-18 structural phase (Fig. 8).

4. Conclusion

A reversible single-crystal-to-single-crystal phase transition is observed for isopropyl-GE ester. The two phases, P-11-18 and P-13-17, are not isostructural, although the same scheme can describe their supramolecular architectures and building
motifs. Similar structural motifs are also observed in the ethyl- and 2-morpholinomethyl-GE esters and GE methanol solvate. Analysis of the crystal packing revealed that the P-11-18 phase resembles the structure of GE methanol solvate. The second phase is isostructural with ethyl-GE ester. The phase transition is related to the larger volume occupied by the ester substituent at higher temperatures. The supramolecular architecture of P-11-18 is not able to accommodate this change. As a consequence, significant rotation of the triterpene molecules in the 1D ribbons and different geometries of the O—H···O interlayer interactions in the P-13-17 compared to the P-11-18 phase are observed.

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References


