# Single Crystal X-Ray Diffraction Structure of a Pseudo-polymorph of (±)-3-(ethoxycarbonyl)-2-(imidazol-1-yl) Propionic Acid (IEPA)

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Received: January 9, 2013Accepted: February 27, 2013Online Published: April 2, 2013doi:10.5539/ijc.v5n2p1URL: http://dx.doi.org/10.5539/ijc.v5n2p1

# Abstract

 $(\pm)$ -3-(Ethoxycarbonyl)-2-(imidazol-1-yl) propionic acid (IEPA) crystallized in two different forms when recrystallized from absolute ethanol (Form I) or water (Form II), respectively. The single crystal X ray diffraction study of Form II unambiguously established that IEPA yielded a pseudopolymorph with a different crystalline packing when crystallized from water. This behaviour is additionally confirmed by Differential Scanning Calorimetry (DSC) and Thermogravimetry (TG).

Keywords: Polymorphism, single crystal X-ray analysis, crystalline packing, thermal analysis

## 1. Introduction

( $\pm$ )-3-(Ethoxycarbonyl)-2-(imidazol-1-yl) propionic acid (IEPA) (Figure 1) is currently the most appropriate extrinsic probe for non-invasive, preclinical measurements of extracellular pH by magnetic resonance <sup>1</sup>H spectroscopic imaging (MRSI) methods (Gil, Zaderenko, Cruz, Cerdán, & Ballesteros, 1994; Pacheco-Torres et al., 2011; Hashim, Zhang, Wojtkowiak, Martinez, & Gillies, 2011). Furthermore, it has been recently proposed as a convenient therapeutic buffer for the treatment of cancer and metastasis (Hashim et al., 2011).

Detailed analyses of crystal structures and intra and intermolecular interactions are essential for the complete characterization and understanding of the functional properties of solids, as well as for the design of new materials (Hilfiker, 2006). We have described previously that IEPA, crystallized in two different forms when it was recrystallized either from absolute ethanol (Form I) or water (Form II) (Ubide-Barreda, Rojas, Martínez-de Paz, Righi, & Ballesteros, 2011). Comparison of powder X-ray diffraction pattern of Form I and that from Form II, showed clearly that the structures obtained in both cases were different.

In order to confirm this interesting feature we have performed here the single crystal X-ray diffraction analysis of Form II. This information will be useful to prepare and characterize improved IEPA formulations for oral administration (Hashim et al., 2011).

## 2. Material and Methods

2.1 Synthesis of (±)-3-(Ethoxycarbonyl)-2-(imidazol-1-yl) Propionic Acid (IEPA)

Synthesis of IEPA was performed from imidazole and diethyl fumarate according to our previous report

(Zaderenko, Gil, Ballesteros, & Cerdán, 1994.)

2.2 X-Ray Data Collection and Structure Refinement

Data collection was carried out at room temperature on a Bruker Smart CCD diffractometer using graphite-monochromated Mo-K $\alpha$  radiation ( $\lambda$ =0.71073 Å) operating at 50 kV and 35 mA. The data were collected over a hemisphere of the reciprocal space by combination of three exposure sets. Each exposure of 20s covered 0.3 in  $\omega$ . The cell parameters were determined and refined by a least-squares fit of all reflections. The first 100 frames were recollected at the end of the data collection to monitor crystal decay, and no appreciable

decay was observed. A summary of the fundamental crystal and refinement data is given in Table 1.

The structure was solved by direct methods and refined by full-matrix least-square procedures on  $F^2$  (SHELXL-97) (Sheldrick, 1997). All non-hydrogen atoms were refined anisotropically.

The hydrogen atoms were included in their calculated positions and refined riding on the respective carbon atoms with the exception of hydrogens H5A and H5B bonded to O5 that were located in a Fourier synthesis and refined riding on the respective bonded atoms.

Further crystallographic details for the structure reported in this paper may be obtained from the Cambridge Crystallographic Data Center, on quoting the depository number CCDC-852977.

Identification code	iepaH2O			
Empirical formula	$C_9H_{14}N_2O_5$			
Formula weight	230.22			
Temperature	296(2) K			
Wavelength	0.71073 Å			
Crystal system	Monoclinic			
Space group	P2(1)/c			
Unit cell dimensions	a = 4.9692(10) Å	= 90°.		
	b = 23.146(5)  Å	= 103.046(4)°		
	c = 10.407(2)  Å	= 90°		
Volume	1166.1(4) Å <sup>3</sup>			
Ζ	4			
Density (calculated)	$1.311 \text{ Mg/m}^3$			
Absorption coefficient	0.108 mm <sup>-1</sup>			
F(000)	488			
Crystal size	0.45 x 0.16 x 0.11 mm	3		
Theta range for data collection	1.76 to 26.00°	1.76 to 26.00°		
Index ranges	-6<=h<=6, -28<=k<=28, -11<=l<=12			
Reflections collected	9449			
Independent reflections	2266 [R(int) = 0.0668]			
Completeness to theta = $26.00^{\circ}$	99.1%			
Refinement method	Full-matrix least-squares on F <sup>2</sup>			
Data/restraints/parameters	2266/0/145			
Goodness-of-fit on F <sup>2</sup>	1.015			
Final R index [I>2sigma(I)]	R1 = 0.0502, wR2 = 0.	R1 = 0.0502, $wR2 = 0.1269$		
R index (all data)	R1 = 0.1196, wR2 = 0.	R1 = 0.1196, wR2 = 0.1594		
Theta range for data collection	1.76 to 26.00°.	1.76 to 26.00°.		
Index ranges	-6<=h<=6, -28<=k<=2	-6<=h<=6, -28<=k<=28, -11<=l<=12		
Reflections collected	9449			
Independent reflections	2266 [R(int) = 0.0668]			
Completeness to theta = $26.00^{\circ}$	99.1%			
Refinement method	Full-matrix least-squares on F <sup>2</sup>			
Data/restraints/parameters	2266/0/145			
Goodness-of-fit on F <sup>2</sup>	1.015			
Final R index [I>2sigma(I)]	R1 = 0.0502, $wR2 = 0.1269$			
R index (all data)	R1 = 0.1196, $wR2 = 0.1594$			

Table 1. Crystal data and structure refinement for  $C_9H_{14}N_2O_5$ 

#### 2.3 Thermal Analysis (TG and DSC)

Thermal analysis was carried out using a Seiko SSC 5200 TG-TA 320 System. A Sample of Form II (about 20 mg) was heated in nitrogen from 30 up to 125 °C (flow rate = 50 mL·min<sup>-1</sup>) with a heating rate of 10 °C·min<sup>-1</sup>, soaked at this temperature for 3 min, then heated up to 160 °C at a heating rate of 0.5 °C·min<sup>-1</sup> and finally from 160 up to 400 °C at a heating rate of 10 °C·min<sup>-1</sup>.

#### 3. Results and Discussion

#### 3.1 Synthesis of IEPA Form II

Polymorphs I and II of IEPA were obtained according to previous reports (Ubide-Barreda, Rojas, Martínez-de Paz, Righi, & Ballesteros 2011).

#### 3.2 Molecular and Crystal Structure of Form II

Bond distances and angles of Form II (Figure 1) are similar to those of Form I described previously (López et al., 1996). Tables 2 and 3 summarize bonds, angles, hydrogen bonds and torsion angles obtained from single crystal X-ray analysis of IEPA Form II.



Figure 1. ORTEP view of IEPA (Form II) showing the atomic numbering; displacement ellipsoids are drawn at the 20% probability level

The IEPA Form II forms intermolecular hydrogen bonds between the carboxylate O1 and the imidazole N3 of a neighbouring molecule with parameters N3–H3 1.06 Å, H3…O1 1.63 Å and N3–H3–O1 169.2° angle. Intermolecular bonding of the adjacent molecules originates the formation of zig-zag chains. This zig-zag arrangement is due to the torsion found in the molecule between the carboxylate and imidazole planes of  $77.3(2)^\circ$ .

The chains are connected by hydrogen bonds involving the water molecules and the second oxygen atom of the carboxylate group with distances  $O5\cdots O2= 2.936(4)$  Å and  $O5\cdots O2^2=2.903(3)$  Å. This interaction is extended along the "a" crystal axis. It becomes important to remark here the importance of the water molecule interactions in the stabilization of the crystal packing of Form II (Figure 2a). No intermolecular interactions are found involving the ester carboxylate oxygen.

Bond	Bond lengths (Å)	Angle		(deg)
N1-C2	1.335(2)	C5-N1-C6	5	124.2(1)
N1-C5	1.378(2)	C2-NI-C6		128.3(1)
N1-C6	1.468(2)	C2-NI-C5		107.5(1)
C2-N3	1.314(2)	NI-C2-N3		109.7(1)
N3-C4	1.370(2)	C2-N3-C4	ŀ	108.2(1)
C4-C5	1.350(2)	N3-C4-C5	5	107.6(1)
C6-C7	1.544(2)	NI-C5-C4		107.0(1)
C6-C10	1.525(2)	NI-C~CIC	)	111.6(1)
C7-O8	1.229(2)	NI-C6-C7		113.1(1)
C7-O9	1.257(2)	C7-C6-CI	0	113.3(1)
C10-C11	1.502(2)	C6-C7-09		111.6(1)
C11-O12	1.202(2)	C6-C7-08		120.5(1)
C11-O13	1.332(2)	08-C7-09		127.8(1)
O13-C14	1.456(3)	C6-C10-C	11	114.0(1)
C14-C15	1.496(5)	C10-C11-	013	110.4(1)
		C10-C11-	012	125.8(2)
		O12-C11-	013	123.8(2)
		C11-OI3-0	CI4	116.4(2)
		OI3-CI4-C	C15	106.2(3)
Hydrogen bonds				
D-HA	d(D-H)	d(HA)	d(DA)	<(DHA)
O(5)-H(5B)O(2)#1	1.05	1.89	2.903(3)	160.7
O(5)-H(5A)O(2)	1.09	1.86	2.936(4)	166.6
N(3)-H(3)O(1)#2	1.06	1.63	2.677(3)	169.2
Symmetry transformations used to generate equivalent atoms:				
#1 x-1,y,z #2 x+1,-y+3/2,z+1/2				

Table 2. Geometric parameter	s (Å,deg) for	r compound	(±)-3-(ethoxycarbonyl)-2-(imi	idazol-1-yl) p	ropionic a	acid
crystallized from water						

# Table 3. Torsion angles [°]

Torsion angles	Degree (°)	Torsion angles	Degree (°)
N(3)-C(4)-C(5)-N(1)	0.5(3)	C(4)-C(5)-N(1)-C(6)	-179.1(3)
N(1)-C(6)-C(7)-O(1)	-4.8(4)	C(8)-C(6)-N(1)-C(2)	134.5(3)
C(8)-C(6)-C(7)-O(1)	120.5(3)	C(7)-C(6)-N(1)-C(2)	-101.1(3)
N(1)-C(6)-C(7)-O(2)	174.0(3)	C(8)-C(6)-N(1)-C(5)	-47.2(4)
C(8)-C(6)-C(7)-O(2)	-60.7(4)	C(7)-C(6)-N(1)-C(5)	77.2(3)
N(1)-C(6)-C(8)-C(9)	-53.8(3)	N(1)-C(2)-N(3)-C(4)	0.0(3)
C(7)-C(6)-C(8)-C(9)	-179.1(3)	C(5)-C(4)-N(3)-C(2)	-0.3(3)
C(6)-C(8)-C(9)-O(3)	-11.8(5)	O(3)-C(9)-O(4)-C(10)	1.9(6)
C(6)-C(8)-C(9)-O(4)	166.6(3)	C(8)-C(9)-O(4)-C(10)	-176.5(4)
N(3)-C(2)-N(1)-C(5)	0.3(3)	C(11)-C(10)-O(4)-C(9)	104.9(6)
N(3)-C(2)-N(1)-C(6)	178.9(2)	C(5)-C(4)-N(3)-C(2)	-0.3(3)
C(4)-C(5)-N(1)-C(2)	-0.5(3)	O(3)-C(9)-O(4)-C(10)	1.9(6)



Figure 2. Crystal packing of Form II. (a) Three-dimensional view of the zig-zag chains (b) View of one zig-zag chain along the "a" axis

#### 3.3 Thermal Analysis (TG and DSC)

Figure 3 shows the thermogravimetric (TG) and differential scanning calorimetry (DSC) curves of Form II. The TG curve of the hydrated form II shows two mass losses. The first one, occurring in a range of 40 °C-70 °C, is 6%. This loss can be attributed to the loss of water by drying, since the sample is wet to prevent structural degradation. The second mass loss, in a temperature range of 70 °C-140 °C reaching 6%, is attributed to the removal of the water molecules of crystallization. However, we have previously reported a mass loss of 3% at 60-100 °C (Ubide-Barreda, Rojas, Martinez-de Paz, Righi, & Ballesteros, 2011). This variation is most likely due to a different degree of humidity in the conservation of the earlier sample and a consequent partial degradation associated to the smaller water loss. The DSC curve shows three different endothermic peaks. The first one at 70 °C, with a heat of 36 kJ/mol, is due to the rearrangement of the molecular structure involving; the breaking of zwitterionic interactions, the rupture of ring interactions within neighboring chains and the evaporation of moisture. The second peak centered at 120 °C, with a heat 15 kJ/mol., is associated with the loss of water molecules within the crystal structure and the melting point process. Finally, the third peak centred at 260 °C is associated to the decomposition leaving a carbonaceous residue of 13%.



Figure 3. TG (solid line) and DSC (dotted line ) thermograms of Form II

#### 4. Conclusions

The results obtained from single crystal X-ray diffraction study of Form II confirm unambiguously that  $(\pm)$ -3-(ethoxycarbonyl)-2-(imidazol-1-yl)propionic acid (IEPA) yields a pseudopolymorph when crystallized from water. The chains in the crystal are connected by hydrogen bonds involving the water molecules and the second oxygen atom of the carboxylate group with distances O5…O2 = 2.936(4) Å and O5…O2'=2.903(3) Å. This interaction is extended along the "a" crystal axis. The presence of water in the solvate is also confirmed by thermogravimetry. The results obtained herein will be useful to prepare and characterize new IEPA formulations for oral administration with improved bioavailability.

#### Acknowledgements

This work was supported in part by grants: CTQ2010-20960-C02-01, S2010/BMD-2349, MICINN-PTA 2010-03023-I to C.U.

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