ÁCIDO 4-METIL HIPÚRICO: UM CASO DE POLIMORFISMO E SOLVATOMORFISMO

4-METHYL HYPPURIC ACID: A CASE OF POLYMORPHISM AND SOLVATOMORPHISM

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RESUMO

O polimorfismo é conhecido como a capacidade de um material sólido existir em mais de uma forma ou estrutura cristalina, com aplicações importantes na preparação de ingredientes farmacêuticos ativos. A caracterização de diferentes polimorfos do metabólito específico do 4-xileno pode contribuir para a indústria química e farmacêutica. O polimorfismo é de particular importância nos processos industriais, onde diferentes propriedades físicas das formas polimórficas podem alterar substancialmente a viabilidade e a qualidade de um produto manufaturado. Isto é particularmente verdade para a concepção e produção de fármacos na indústria farmacêutica, uma vez que as propriedades físicas variáveis entre diferentes polimorfos podem afetar o prazo de validade e a durabilidade, a solubilidade, bem como a biodisponibilidade e o fabrico do fármaco. A caracterização de cristalização, espectroscopia e difração de raios-X de dois polimorfos e um solvatomorfo de ácido 4-metil-hipúrico são apresentados. Estes compostos cristalizam em diferentes sistemas cristalinos. O polimorfo I (4mH-I) cristaliza em uma célula ortorrômbica com o grupo espacial $P2_12_12_1$. Polimorfo II (4mH-II) cristaliza em um grupo de espaço monoclinico $P2_1/c$. O Solvatomorfo (4mH-S) cristaliza em uma célula P-1 triclinica. Todos os polimorfos cristalizam em forma neutra. O empacotamento cristalino dos três compostos é governado por interações intermoleculares de ligações de hidrogênio do tipo $N-H \cdots O$ e $O-H \cdots O$ formando redes tridimensionais.

Palavras-chave: Polimorfismo, solvatomorfismo, aminoácidos N-acílicos, difração de raios X

ABSTRACT

Polymorphism is known as the ability of a solid material to exist in more than one form or crystal structure, with important applications in the preparation of active pharmaceutical ingredients. Characterization of different polymorphs of the specific metabolite of 4-xylene can contribute to the chemical and pharmaceutical industry. Polymorphism is of particular importance in industrial processes, where different physical properties of polymorphic forms can substantially alter the viability and quality of a manufactured product. This is particularly so for the design and production of drugs in the pharmaceutical industry, as varying physical properties between different polymorphs can affect shelf life and durability, solubility, as well as bioavailability and manufacturing of the drug. The crystallization, spectroscopic and X-ray diffraction characterization of two polymorph and one solvatomorph of 4-methylhippuric acid are presented. These compounds crystallizes in different crystalline systems. Polymorph I (4mH-I) crystalize in an orthorhombic cell with space group $P2_12_12_1$. Polymorph II (4mH-II) crystallizes in a monoclinic space group $P2_1/c$. Solvatomorph (4mH-S) crystallizes in a triclinic P-1 cell. All polymorphs crystallize in neutral form. The crystal packing of the three compounds are governed by hydrogen bonds intermolecular interactions of the type $N-H \cdots O$ and $O-H \cdots O$ forming tridimensional networks.

Keywords: Polymorphism, solvatomorphism, N-acyl amino acids, X-ray diffraction

1. INTRODUCTION

Amino acids and their derivatives are the fundamental units of proteins and peptides, which realize diverse functions participating in most biological processes and constituting fundamental structures in living beings. An interesting group of amino acids derivatives are the N-acyl amino acids, which are obtained from the conjugation of the amino acid with an acyl group, which is bound through the amino group (Wakamatsu *et al.*, 1971). The most important of this group of compounds is hippuric acid, or N-benzoylglycine acid, since it is the main metabolite of toluene and its determination in human urine is indicative of toluene contamination. In the same way, methylhippuric acid derivatives (*ortho*, *meta* and *para*) are specific metabolites of methylated xylene derivatives. Main studies of hippuric and methylhippuric acid focus their identification and quantification by gas chromatography or high performance liquid chromatography resolution as direct indicators of toluene levels and xylene isomers in urine samples, respectively. Therefore, its determination to know the levels of contamination is essential, especially in the workers of the chemical industry (Sperlingová *et al.*, 2007; Antunes *et al.*, 2013). Thus, quantification of hippuric and methylhippuric acid in urine is actually used as a diagnostic marker of exposure to toluene and xylene. Figure 1 shows the hippuric and 4-methylhippuric structural diagram.

Figure 1. Diagram of N-acyl amino acids hippuric and 4-methylhippuric.

On the other hand, polymorphism is known as the ability of a solid material to exist in different molecular arrangements and/or different molecular conformations (McCrone, 1965; Bernstein, 2002), while solvatomorphism is used to denote other crystal variations where the crystal structure differ in their elemental composition through the inclusion of one or more molecules of solvent (Brittain, 2010). The crystallographic aspect of polymorphism and solvatomorphism are part of an interesting field in materials science researches (Lee *et al.*, 2011; Cruz and Bernstein, 2014). Polymorphs of the

same compound exhibit different physical and chemical properties, such as: solubility, melting point, color, among others, properties of nonlinear optics and magnetic properties, with important applications in the pharmaceutical industry, food, materials and physicochemical research in the solid state (Park *et al.*, 2005), and crystallization conditions (polarity solvents, pH, agitation, pressure changes, temperature, etc.) play a key role in the preparation of polymorphs (Bond, 2009).

In our laboratory we are interested in the structural study of natural and non-natural amino acids and their derivatives (Delgado *et al.*, 2007; 2012; 2015; 2016; 2019; Seijas *et al.*, 2010; Guillen *et al.*, 2015; Mora *et al.*, 2017; Fernández *et al.*, 2018; Chacón *et al.*, 2018) and previously reported the crystallization, spectroscopic and powder diffraction patterns of the isomers *ortho*, *meta* and *para*-methyl hippuric acids (Guillen *et al.*, 2015; Delgado *et al.*, 2016). In this work it is presented the structural characterization of two polymorphs and one solvatomorph found in the crystallization of the N-acyl amino acid 4-methylhippuric.

2. MATERIALS AND METHODS

2.1. Synthesis

In this work was used 4-methylhippuric acid, $C_{10}H_{11}NO_3$, Aldrich 98% with analytical grade. 1.55 mmol (0.030 g) of the acid were weighed and dissolved, separately in deionized water (polymorph-I 4mH-I), methanol: water 1: 1 (polymorph-II 4mH-II) and methanol: ethanol 1: 1 plus 0.5mL of a solution of 0.1M sodium hydroxide to adjust the pH to 9 (solvatomorph 4mH-S). The solutions were refluxed with constant stirring for 1 hour. Suitable crystals were obtained, after several weeks, by slow evaporation of solvents. The crystals formed are colorless with optimal regular form for study by powder and single-crystal X-ray diffraction. Melting points were measured in an Electrothermal model 9100 apparatus.

2.2. Fourier transform Infrared spectroscopy

The Fourier transform infrared spectroscopy (FT-IR) absorption spectra were obtained as KBr pellet using a Perkin-Elmer 1600 spectrometer (Perkin-Elmer, USA).

2.3. Powder and single-crystal X-ray diffraction

A small quantity of each sample was ground mechanically in an agate mortar and pestle. The resulting fine powders were mounted on a flat zero-background holder covered with a thin layer of petroleum jelly. The X-ray powder diffraction data was collected at room temperature, in θ/θ reflection mode using a Siemens D5005 diffractometer equipped with an X-ray tube (CuK α radiation: $\lambda = 1.54059 \text{ \AA}$; 40kV, 30 mA). The data were scanned from $5-50^\circ 2\theta$, with a step size of 0.02° and counting time of 10 s. Silicon was used as an external standard.

The single-crystal X-ray diffraction data were measured on a Bruker Smart Apex II CCD diffractometer equipped with MoK α radiation ($\lambda = 0.7076 \text{ \AA}$). The data was corrected for absorption and polarization effects.

3. RESULTS AND DISCUSSION:

Melting points measured were: 4mH-I 163-165 $^\circ\text{C}$, 4mH-II 160-161 $^\circ\text{C}$ and 4mH-S 150-152 $^\circ\text{C}$. Figure 2 shows the FT-IR spectra of the three crystalline forms of 4-methylhippuric acid (4mH-I, 4mH-II and 4mH-S). From these, the absorption bands characteristic of the molecular skeleton of the N-acyl amino acids were identified and assigned. In all three cases tension signals of N-H and C=O bonds of the amide group at 3354 cm^{-1} and 1653 cm^{-1} for polymorph I (4mH-I) and polymorph II (4mH-II) are highlighted. In the solvatomorph (4mH-S) this signal undergoes a bathochromic displacement at 3378 cm^{-1} for the N-H and hypsochromic signal at 1636 cm^{-1} for the C=O bond. On the other hand, the tension and flexion signals of the OH bond of the carboxylic acid group (COOH) appear in the region of $3200-2750 \text{ cm}^{-1}$ and 1418 cm^{-1} , respectively for 4mH-I, $3138-2770 \text{ cm}^{-1}$ and 1419 cm^{-1} for 4mH-II and in the case of 4mH-S, two signals appear, the first in the range $3020-2850 \text{ cm}^{-1}$ and 1417 cm^{-1} associated to the O-H bonds of the acid group and the second broadband and of greater intensity in the region $3650-3150 \text{ cm}^{-1}$ indicative of the presence of water molecules of crystallization. With respect to the voltage signal of the C=O bond of the acid group it appears at 1741 cm^{-1} for 4mH-I, while at 4mH-II it moves bathochromically at 1748 cm^{-1} and for 4mH-S it moves hypsochromically at 1683 cm^{-1} . The appearance of these bands in all three cases suggests that the N-acylamino acid is found in neutral form in all its crystalline forms.

The X-ray powder patterns of the three polymorphs indicates the presence of single phase and different symmetries in each case, as

shown in Figure 3. The indexing of the patterns was performed using the program DICVOL04 (Boultif and Louer, 2004). Polymorphs I and II crystallize in orthorhombic and monoclinic cells, respectively. The solvatomorph crystallizes in a triclinic cell. The unit cell parameters are summarized in Table 1 together with the indexing figures of merit M_N (de Wolf, 1968) and F_N (Smith and Snyder, 1979).

Table 1. Unit cell parameters obtained from pattern indexing of 4mH-I, 4mH-II and 4mH-S

	4mH-I	4mH-II	4mH-S
<i>a</i> (Å)	5.178(2)	9.640(2)	6.948(2)
<i>b</i> (Å)	8.290(2)	8.662(2)	8.286(3)
<i>c</i> (Å)	22.270(4)	23.691(5)	20.107(2)
α (°)			89.93(1)
β (°)		94.27(2)	83.32(2)
γ (°)			75.52(1)
<i>V</i> (Å ³)	956.0(1)	1972.7(1)	1054.53(5)
<i>M</i> ₍₂₀₎	31.7	30.3	38.1
<i>F</i> ₍₃₀₎	45.0	37.9	49.1
	(0.0049, 90)	(0.0085, 90)	(0.0058, 55)

The crystal structures, using single-crystal diffraction data, were determined using the SHELXS program (Sheldrick, 2008) and refined by full-matrix least squares calculations using the SHELXL program (Sheldrick, 2015). All atoms were placed in calculated and treated positions using a rigid model with distances CH 0.96.0.98 Å and Uiso (H) = 1.2 Ueq (C)], OH 0.82 Uiso (H) = 1.2 Ueq (O)], NH 0.86 Å and Uiso (H) = 1.2 Ueq (N)]. The crystal structure analysis of three polymorphs are in process and will be published elsewhere. Table 2 summarizes the crystallographic data and structure refinement parameters for each polymorph. Table 3 shows some geometric parameters such as distances, bond angles and torsion angles of 4-methylhippuric acid in the different crystalline forms.

All polymorphs crystallize in neutral form. The bond distances O1-C1 greater than the distances O2-C1 indicate the position of the hydrogen of the OH group and corroborate the double bond in the distance O2-C1.

Polymorph I (4mH-I) crystallizes in an orthorhombic cell with space group $P2_12_12_1$. The crystal packing shows a network stabilized by hydrogen bonds. One of the type N--H...O linking the nitrogen of the amide with the carbonyl group of the amide of another acid molecule, which can

be described with the graph-set notation C(5) (Etter, 1990). Another hydrogen bond is the type O--H...O, which allows the formation of 20 atoms macrocycle where four donor and acceptor groups form a ring described by the graph $R^4_4(20)$ (Figure 4).

Polymorph II (4mH-II) crystallizes in a monoclinic cell with space group $P2_1/c$ and two molecules in the unit cell. The geometric arrangement of these molecules in crystal packing generates dimers acid-amide in the *ca* plane, governed by intermolecular hydrogen bonds of the type O--H...O forming cyclic dimers described with the graph $R^2_2(12)$. These dimers are bound through hydrogen bonds of the type O--H...N in the [100] direction. The second dimer is formed by the interaction of N--H...O hydrogen bonding type along the *b* axis and described by chains with graph-set $C^2_2(10)$ (Figure 5).

Solvatomorph (4mH-S) crystallizes in a triclinic cell with space group $P-1$. The asymmetric unit comprises two molecules of amino acid and two water molecules. Both molecules are joined to each other by bifurcate N--H...O hydrogen bonding interactions forming a dimer molecule A-molecule-B in the *ac* plane, this link can be described by the graph $R^1_2(7)$. The water molecules play a fundamental role in the stabilization of the acid molecules in the solid state, since it acts as a bridge between the dimers along the *b*-axis where N--H...O hydrogen bonds are involved (Figure 6).

4. CONCLUSIONS:

Conformational polymorphs of 4-methylhippuric acid were obtained by varying the polarity and pH conditions of the solvent medium. They crystallize in orthorhombic (polymorph I), monoclinic (polymorph II) and triclinic (solvatomorph) cells. The crystal packing for the three polymorphs of 4-methylhippuric acid are stabilized by hydrogen bonds of the type N--H...O and O--H...O. In the solvatomorph the water molecules play a fundamental role acting as a bridge between the acid dimers.

Characterization of different polymorphs of 4-methylhippuric acid, the specific metabolite of 4-xylene, can contribute to the chemical and pharmaceutical industry, where different physical properties of polymorphic forms can substantially alter the viability and quality of a manufactured product.

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Figure 2. FT-IR spectra of the polymorphs I, II and solvatomorph of 4-methylhippuric acid

Figure 3. X-ray powder diffraction patterns for polymorphs I, II and solvatomorph of 4mH acid

Table 2. Crystallographic data for the 4-methylhippuric polymorphs

	polymorph-I (4mH-I)	polymorph-II (4mH-II)	solvatomorph (4mH-S)
Formula	C ₁₀ H ₁₁ NO ₃	C ₁₀ H ₁₁ NO ₃	C ₁₀ H ₁₁ NO ₃ ·H ₂ O
Weight (uma)	193.20	193.20	211.20
mp (°C)	163-165	160-161	150-152
System	orthorhombic	monoclinic	triclinic
Space group	P2 ₁ 2 ₁ 2 ₁ (N°19)	P2 ₁ /c (N°14)	P-1 (N°2)
a (Å)	5.1732(18)	9.6313(1)	6.947(3)
b (Å)	8.286(3)	8.6587(1)	7.857(3)
c (Å)	22.264(8)	23.703(3)	20.114(7)
α (°)	-	-	89.948(9)
β (°)	-	94.270(3)	83.351(1)
γ (°)	-	-	75.535(9)
V (Å ³)	954.35(6)	1971.2(4)	1055.5(7)
Z	4(1)	8(2)	4(2)
ρ _{calc} (g/cm ³)	1.345	1.302	1.316
Uniq. refl. (Rint)	770 (0.026)	4038 (0.072)	4303(0.317)
R(F ²) [I > 2σ(I)]	0.0259	0.0803	0.1192
wR(F ²) [I > 2σ(I)]	0.0735	0.1965	0.2375
S	1.02	1.11	1.01

Table 3. Bond distances (Å), angles and torsion (°) in polymorphs 4mH-I, 4mH-II and 4mH-S

4-Methyl hippuric acid	4mH-I	4mH-II		4mH-S	
		Molecule A	Molecule B	Molecule A	Molecule B
O1-C1	1.324(5)	1.322(4)	1.320(5)	1.303(1)	1.307(9)
O2-C1	1.197(5)	1.210(4)	1.198(4)	1.231(9)	1.213(9)
O3-C3	1.229(5)	1.248(5)	1.246(4)	1.237(1)	1.250(1)
N1-C2	1.437(5)	1.437(5)	1.442(5)	1.447(9)	1.457(1)
N1-C3	1.345(5)	1.337(5)	1.333(4)	1.334(9)	1.323(1)
C2-N1-C3	122.3(3)	120.6(3)	120.9(3)	122.2(6)	121.8(7)
O1-C1-O2	123.8(3)	124.1(3)	124.3(4)	122.3(7)	123.4(7)
C3-N1-C2-C1	83.4(4)	-68.5(4)	-77.9(4)	-81.7(9)	-71.9(9)
C2-N1-C3-C4	-176.2(3)	178.6(3)	-170.7(3)	177.9(6)	177.7(6)
O3-C3-C4-C5	-8.9(6)	1.9(5)	-29.1(5)	-24.3(1)	-18.7(1)