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Interactions between Isoniazid and α-Hydroxycarboxylic Acids †

Raquel Álvarez-Vidaurre, Alfonso Castiñeiras, Isabel García-Santos * and Rocío Torres-Iglesias

Department of Inorganic Chemistry, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain; email1@gmail.com (R.Á.-V.); email2@gmail.com (A.C.); email1@gmail.com (R.T.-I.)

* Correspondence: isabel.garcia@usc.es; Tel.: +34-881-814-956

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Abstract: The present study refers to the preparation of isonicotinic acid hydrazide (isoniazid, **inh**) co-crystals with two α-hydroxycarboxylic acids. The interaction of glycolic acid (**H2ga**) or DL-mandelic acid (**H2ma**) resulted in the formation of co-crystals or salts of composition (inh)·(H_2ga) (**1**) and [Hinh]$^+$[Hma]$^-$·(H_2ma) (**2**) when reacted with isoniazid. An N′-(propan-2-ylidene)isonicotinic hydrazide hemihydrate, (pinh)·1/2(H_2O) (**3**), was also prepared by condensation of isoniazid with acetone in the presence of glycolic acid. The prepared compounds were well characterized by elemental analysis, and spectroscopic methods, and their three-dimensional molecular structure was determined by single crystal X-ray crystallography. Hydrogen bonds involving the carboxylic acid occur consistently with the pyridine ring N atom of the isoniazid and its derivatives. The remaining hydrogen-bonding sites on the isoniazid backbone vary based on the steric influences of the derivative group. These are contrasted in each of the molecular systems.

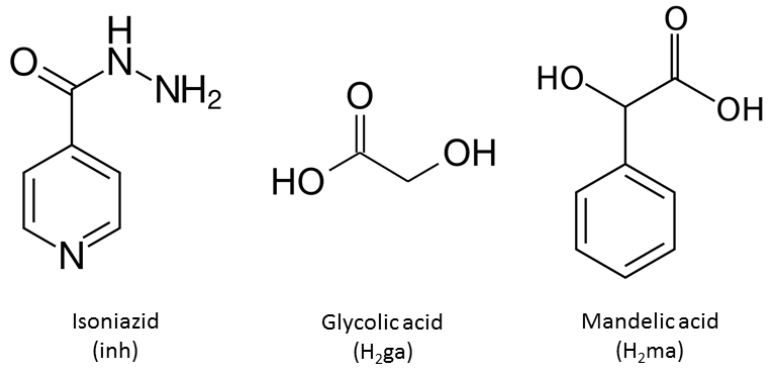
Keywords: isoniazid; cocrystals; glycolic acid; DL-mandelic acid; crystal structure

1. Introduction

As an important part of supramolecular chemistry, crystal engineering is the subject of continuous research in solid and materials science. Rapid development in this field has revealed the use of a variety of organic components with specific functional groups to create supramolecular arrays through the coordination of metals or non-covalent forces, presenting interesting structures and useful properties. In this context, numerous recent examples of multicomponent crystals are known, the assembly of which is driven by non-covalent interactions, mainly hydrogen bonding with or without charge assistance [1]. Acid-base binary cocrystals is an important technological topic in pharmaceutical science that has attracted scientific and pharmaceutical interest in recent decades due to their potential ability to modify important properties of active pharmaceutical ingredients (APIs) such as solubility, dissolution rate, bioavailability, hygroscopicity, and/or thermal stability [2]. Furthermore, the formation of these multicomponent crystals does not lead to changes in the nature of the API, unlike the situation observed during salt formation, where the API must protonate or be protonated [3].

In this field of research, isonicotinic acid hydrazide (isoniazid, **inh**) (Scheme 1) is an important API that, among others, is applied in combination with rifampicin, pyrazinamide and ethambutol for the treatment of tuberculosis, which are known as fixed-dose combinations [4]. Furthermore, the reaction of **inh** with ketones is used to modify or improve their molecular efficacy at the biological level [5]. For example, in the compound obtained with 2-propanone, an increase in activity against Mycobacterium tuberculosis is observed with respect to **inh** [6]. From the point of view of crystal

engineering, **inh** represents interest in the design of cocrystals, since it has an N-pyridine, which is well known for forming pyridine-carboxylic acid heterosynthons with carboxylic acids, and a carbohydrazide group which can form a range of homo and heterosynthons with other functional groups [7,8]. Therefore, isoniazid can be considered an ideal molecule for studies in the field of pharmaceutical co-crystals.



Scheme 1. Molecular structures of the formers/coformers.

As possible coformers of salts or co-crystals against **inh**, we have considered α-hydroxycarboxylic acids, glycolic (**H2ga**) and DL-mandelic (**H2ma**) (Scheme 1) [9,10]. From the point of view of hydrogen bonding, each of them contains three acceptor oxygen atoms and two donor O-H groups. Both participate in many biochemical processes and have widespread applications both in biological systems and in industry. Thus, glycolic acid, a common component of sugarcane juice and other foods, has an important role in photosynthesis and plant respiration and is a known precursor to oxalate in humans [11], while mandelic acid is a useful precursor to various drugs, for example, homatropine and cyclandelate, which are esters of mandelic acid, and it is also known to have antibacterial properties [12], and has been studied in the preparation of antitumor compounds [13]. Taking into account the previous considerations, the main objective of this work has been the design, preparation, characterization of the physicochemical properties, and identification of recurrent supramolecular patterns within a new set of multicomponent pharmaceutical crystals that involve isoniazid with glycolic and DL-mandelic acids as coformers (Scheme 1).

2. Materials and Methods

Glycolic acid, DL-mandelic acid and isoniazid were purchased from Sigma-Aldrich. Commercially available solvents were used as received without further purification. Compounds were prepared by co-crystallization via solvent-drop grinding: Stoichiometric amounts of inh with H2ga or H2ma were ground with a mortar and pestle for ca. 5–7 min with the addition of 10 μL of solvent per 50 mg of co-crystal formers. The resulting solutions were left to evaporate slowly under ambient conditions. The single crystals of (inh)·(H2ga) (1), $[Hinh]^{+}[Hma]^{-}$·(H2ma) (2) and (pinh)·1/2(H_2O) (3), suitable for X-ray diffraction studies, were obtained in 2–15 days from ethyl acetate or cyclohexane, water and acetone solutions, respectively. Microanalyses (C, H and N) were carried out using a Carlo-Erba 1108 elemental analyser. FT-IR spectra were recorded from KBr pellets over the range 4000–400 cm^{-1} on a Bruker IFS-66v spectrometer. For X-ray analysis, intensity data were collected at 100 K on a Bruker X8 KappaAPEXII diffractometer. Structures were solved by direct methods followed by difference Fourier calculations, and were refined by a full-matrix least-squares procedure using SHELXLTL. The structures were deposited at the Cambridge Crystallographic Data Centre with CCDC Nos. 2041154-2041156, respectively.

3. Results and Discussion

The three crystals were obtained from the crystallization of solutions prepared by reacting the isoniazid with glycolic or mandelic acids in a molar ratio 1:1. Although the X-ray diffraction data were taken at 100 K, solid handling was always done at room temperature.

The cocrystallization processes have been carried out considering the pK_a of isoniazid, and coformers, the glycolic and DL-mandelic acids. Isoniazid has three pK_a values: 1.8 based on hydrazine nitrogen, 3.6 based on pyridine nitrogen and 10.8 based on acidic group [14]. This makes the pyridine the more basic of the two, and in the presence of a carboxylic acid group, is protonated first. The pK_a of the glycolic acid molecule is 3.2 [15], giving a value of $\Delta pK_a = pK_{a(base)} - pK_{a(acid)} = 0.4$ for the combination of the acid with the pyridine group, and of −1.4 of the acid with the hydrazine nitrogen. Regarding mandelic acid, the pK_a is 3.4 and now the values of ΔpK_a are 0.2 and −1.6, respectively. This range has been given by previous researchers as a rule of thumb where the result cannot be easily predicted. [16]. In general, a $\Delta pK_a > 3$ will be expected to form a salt, while a $\Delta pK_a < 0$ almost certainly results in a neutral co-crystal. It is the narrow region between 0 and 3 that does not allow for accurate predictions [17]. The ΔpK_a for the acid and amine combination is −1.4/−1.6, which would predict that no proton transfer should occur since the value is less than 0. However, in the case of DL-mandelic acid, crystallographic results confirm that proton transfer occurs. A caveat to the calculations is that the reported pK_a for the two molecules, isoniazid and glycolic acid or DL-mandelic acid, depends on the solvent used and its polarity. While compound **1** has been obtained in ethyl acetate (ϵ = 6.2) or cyclohexane (ϵ = 2.02), compound **2** has crystallized from water (ϵ = 80). Furthermore, it should be noted that a comprehensive study of 6465 crystalline compounds containing ionized (A^+B^-) and non-ionized (AB) acid-base pairs in the CSD, at $1 < \Delta pK_a < 2$ values, the occurrences of AB and A^+B^- are practically the same [17].

Structural Description and Supramolecular Analysis.

The isoniazid−glycolic acid cocrystal (**1**) crystallizes in the monoclinic $P2_1/n$ space group with unit cell dimensions of a = 3.8930(3) Å, b = 9.9754(5) Å, c = 23.6410(12) Å, β = 92.480(3)° and V = 917.22(10) Å^3. The asymmetric unit contains one isoniazid molecule and one glycolic acid molecule (Figure 1a). Glycolic acid is hydrogen-bonded to isoniazid pyridine N through O−H···N. The angle between the carboxyl group plane and the pyridyl ring plane is 4.9°. A weak pyridyl−glycolic acid C−H···O hydrogen bond results in an $R_2^2(7)$ ring motif (Figure 1b). This synthon has also been observed in other isoniazid cocrystals with carboxylic acids. Furthermore, each isoniazid molecule is linked by hydrogen bonding to two other nearest neighbor molecules through N-H···O, as donor and acceptor, respectively, forming zig-zag chains parallel to the "b" axis which, in turn are linked through head-to-tail glycolic acid molecules, by two new hydrogen bonds O-H···N and O-H···O, so a sequence … H$_2$ga-inh-H$_2$ga-inh … is established along the "c"-axis.

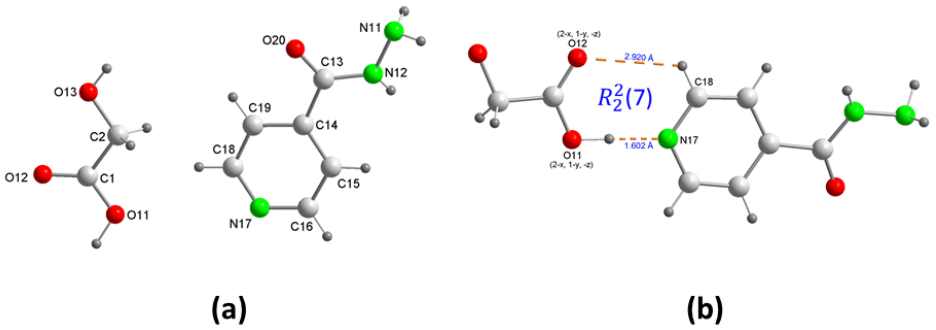


Figure 1. (**a**) Asymmetrical unit of (inh)·(H$_2$ga) (**1**) showing the atom-numbering scheme, and (**b**) detail of $R_2^2(7)$ ring motif.

Isoniazid−DL-Mandelic Acid Salt (**2**). Cocrystallization, using a 1:1 ratio, of racemic DL-mandelic acid and achiral isoniazid by mechanochemistry and liquid-assisted grinding, in the presence of

water, result in the formation of a salt that crystallizes in the monoclinic $P2_1/c$ space group with unit cell dimensions of a = 5.6049(5) Å, b = 24.388(3) Å, c = 15.1471(16) Å, β = 92.025(7)° and V = 2069.2(4) Å^3. The asymmetric unit of **2** contains one isoniazideammonium cation $[Hinh]^+$, one DL-mandelato anion, and one DL-mandelic acid molecule of solvation. Figure 2a shows the crystal structure of **2**. In the unusual protonated isoniazid cation, the DL-mandelate anion transfers its proton to the hydrazine nitrogen giving rise to a robust hydrogen bond N^+–H···O^- that is supported by a N–H···O^- and a C–H···O^- hydrogen bonds, resulting in $R_2^2(7)$ and $R_2^1(7)$ rings, respectively (Figure 2b). The molecule of neutral DL-mandelic acid of crystallization is hydrogen bonded to the N pyridine of the isoniazidammonium cation through O–H···N. The angle between the plane of the carboxyl group and the plane of the pyridyl ring is 9.23°. A weak hydrogen bond C–H···O, pyridyl-mandelic acid, results in a $R_2^2(7)$ ring motif (Figure 2b).

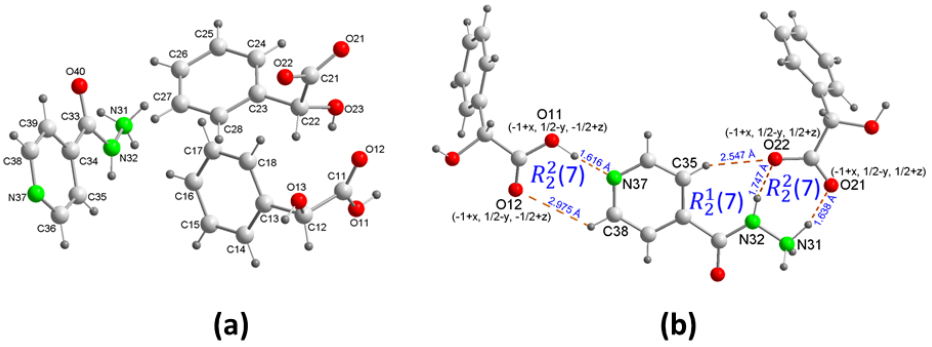
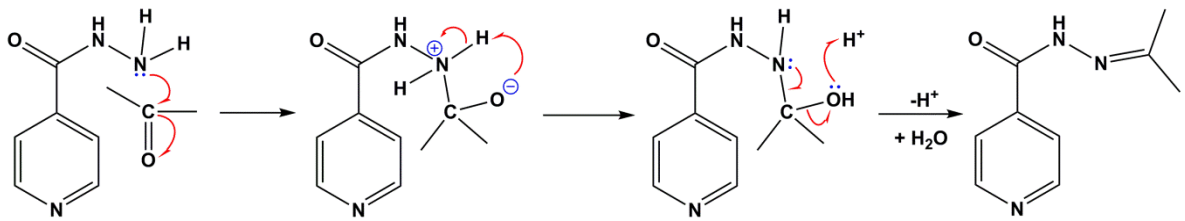


Figure 2. (**a**) Asymmetrical unit of $[Hinh]^+[Hma]^-(H_2ma)$ (**2**) showing the atom-numbering scheme, and (**b**) detail of rings motif.

From a crystal engineering viewpoint, **inh** is well known to form pyridine–carboxylic acid heterosynthons with carboxylic acids. According to a recent study, 39 structures of **inh** cocrystals have been identified with coformers containing COOH groups, representing approximately 40% of all **inh** structures deposited in the CSD, where the acid-pyridine synthon is the most recurrent and is present in approximately 87% of structures [3].

N′-(propan-2-ylidene)isonicotinichydrazide hemihydrate, (**3**), was prepared by adapting the crystallization method described in the literature [18]. The product was obtained by the cocrystallization of isoniazid and glycolic acid from acetone at room temperature. Slow evaporation of the solvent under ambient conditions produced single crystals of (**3**).

The process consists of a one-pot synthesis, with covalent modification occurring in situ, where **inh** is reacted with acetone, or in general with molecules containing ketone or aldehyde functional groups (RC=O), so that the NH_2 group of the carbohydrazide moiety undergoes a condensation reaction and replaces the two H atoms with alkyl groups to form isonicotinohydrazides (Scheme 2). This technique is similar to what has been called "covalence-assisted supramolecular synthesis", with the difference that in this case a hemihydrate has been obtained instead of a cocrystal with a glycolic acid molecule as coformer, as expected [19].



Scheme 2. Mechanism for the formation of N′-(propan-2-ylidene)isonicotinohydrazide from isoniazid.

Compound **3** crystallizes in the orthorhombic, *Aba2* space group and unit cell dimensions a = 18.8351(5) Å, b = 12.6568(4) Å, c = 8.0435(3) Å, and V = 1917.51(11) Å^3. The asymmetric unit of **3** contains one N′-(propan-2-ylidene)isonicotinichydrazide molecule and half a molecule of water. Figure 3a shows the crystal structure of **pinh**.

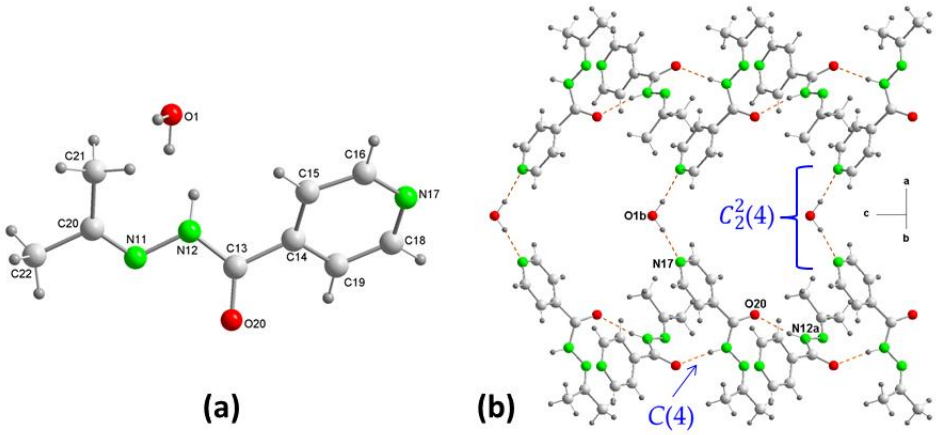


Figure 3. (**a**) Asymmetrical unit of (pinh)·1/2(H_2O) (**3**) showing the atom-numbering scheme, and (**b**) hydrogen-bonding (dashed lines) motifs.

Symmetry positions: a, 1/2 − x, y, −1/2 + z; b, 1/2 − x, y, 1/2 + z

The distances and angles within the **pinh** are as expected. The crystal structure of **3** shows a substantial change in the pattern and packing of hydrogen bonds with respect to the structures of **1** and **2**. The replacement of the two hydrazine hydrogen atoms by the propylidene group removes most of the functionality of the hydrogen bonds of isoniazid. Likewise, the presence of half a molecule of water in the crystalline structure instead of a COOH group of a carboxylic acid, when this is a coformer in a cocrystal with the modified isoniazid, gives rise to a different packing. The crystal structure consists of a 1D network of $C(4)$ chains formed by homomeric hydrogen bonding of the amide group, i.e., N12–H12A···O20, linking symmetrically related **pinh** molecules to form chains along the "c"-axis (Figure 3b). Such chains have been observed in N′-(propan-2-ylidene)isonicotinohydrazide cocrystals with carboxylic acids [18,19]. Furthermore, the characteristic carboxylic acid-pyridine heterosynton of $R_2^2(7)$ graph-set motif [18,19], that is formed between the COOH group and the N′-(propan-2-ylidene)isonicotinohydrazide molecule in carboxylic acid cocrystals with the modified isoniazid, in (**3**) is replaced by a water-pyridine hydrogen bond. Thus, each water molecule acts as a bridge joining two chains of **pinh** molecules to form a 2D structure parallel to the "ac" plane, where a graphic set $C_2^2(4)$ is present (Figure 3b).

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Conflicts of Interest: The authors declare no conflict of interest.

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