Crystal Chemistry of the Antibiotic Doripenem

VALENTINA COLOMBO,[1] NORBERTO MASCIOCCHI,[2] GIOVANNI PALMISANO[2]

[1]Dipartimento di Chimica, Università degli Studi di Milano, Milano 20133, Italy
[2]Dipartimento di Scienza ed Alta Tecnologia, Università dell'Insubria, Como 22100, Italy

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ABSTRACT: Doripenem, an ultrabroad spectrum-injectable antibiotic belonging to the wide class of carbapenem beta-lactams, is commonly marketed as powders of a pure monohydrate phase. Here, we have selectively prepared another hydrated phase (a dihydrate) and determined the crystal structure of both forms by state-of-the art powder diffraction methods. Both phases crystallize in the monoclinic $P2_1$ space group, and, to some extent, are structurally related. Moreover, by using variable temperature diffractometric analyses, we also discovered a crystalline anhydrous form of doripenem, the structure of which (with two crystallographically independent molecules in the monoclinic $P2_1$ space group) remains so far unknown. The thermal interconversion among these phases was further studied by thermogravimetry, differential scanning calorimetry, and thermodiffractometric analyses, and their (metric or stereochemical) mutual relations fully analyzed. The complete structural characterization of the two hydrated phases allows the use of accurate whole pattern profile-fitting procedures for quantitative analyses of these drugs in polycrystalline, or even amorphous, matrices, opening the way to industrial process control and legal protection. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci
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INTRODUCTION

The term polymorphism comes from the Greek word, πολύς, which means many, and μορφή, which means shape, and describes the ability of a substance to exist as two or more crystalline forms. This means that polymorphic solids of the same chemical compound differ in internal solid-state structure and, therefore, possess different chemical and physical properties, including packing, thermodynamic, spectroscopic, kinetic, and mechanical properties.[1,2] Specifically different properties are also manifested by solids containing the same molecules in the presence of additional entities (such as water or other crystallization solvents, additives, or different counterions), which, *per se*, should be considered pseudopolymorphic systems, solvates, salts, or cocrystals. Significantly, in the pharmaceutical industry, these properties can have a direct impact on drug substance processability, drug product manufacturing, and product quality and performances, and even on drug stability, dissolution, and bioavailability.[3] This is the reason why, nowadays, the study of polymorphism and crystallization of active pharmaceutical ingredients (APIs) is highly important: from the discovery of new crystal phases that can ameliorate marketing of the drug, with better formulations, to the swelling up of the patent panorama with significant economic consequences.[4] Indeed, the knowledge of the solid-state properties and their relationships to the crystal structure of the multiple forms of an API are nowadays an integral part of drug development. This is particularly true when a pharmaceutical company markets an API in a solid-state form,[5] as in the vast majority (>90%) of the cases. Accordingly, the scientific literature on drug polymorphism and related phenomena is steadily increasing, but the complete structural information of these pharmaceutical compounds is often lacking, because of the difficulty in growing single crystals of metastable phases from solution (preventing structure determination by conventional X-ray diffraction methods). However, when microcrystalline powders are available, the crystal chemistry of these drugs can be unraveled by state-of-the-art X-ray powder diffraction (XRPD) methods, as successfully carried out in the recent past by us[6–10] and others.[11,12]

Among the antimicrobials structurally related to penicillin, carbapenems have recently attracted the attention of many.[13] Carbapenems have reached a very advanced stage with several worldwide marketing approvals (*e.g.*, olivanic acid, thienamycin, meropenem, imipenem, ertapenem, doripenem, panipenem, and biapenem) and became one of the fast-growing classes in the market of antibiotics. Particularly, doripenem, (Scheme 1) is the newest agent in this class. Doripenem, (+)-(4*R*,5*S*,6*S*)-3-[[(3*S*,5*S*)-5-[[(aminosulfonyl)amino]methyl]-3-pyrrolidinyl]thio]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-carboxylic acid (S-4661), (CAS registry No.: 364622-82-2) has been developed and launched (as monohydrate) by Shionogi Research Laboratories, Shionogi & Co., Ltd. (Osaka, Japan), under the brand name Finibax® in 2005 for intravenous use. It has been later approved by the US FDA (October 2007) and authorized in the EU since July 2008 and marketed, outside Japan, as Doribax®. Significantly, the presence of a 1β-methyl in the carbapenem unit in doripenem enhances its stability to AmpC and extended spectrum β-lactamases (ESBLs), and it is currently used for the treatment of complicated intra-abdominal infections and severe urinary tract infections.[14]

A few studies regarding qualitative and quantitative methods, applied to identify and quantify doripenem in the bulk as well as within pharmaceutical formulations, are found in the literature.[15] However, related to its solid-state structure, little

Correspondence to: Valentina Colombo (Telephone: +39-02-50314450; Fax +39-02-50314454; E-mail: valentina.colombo@unimi.it)
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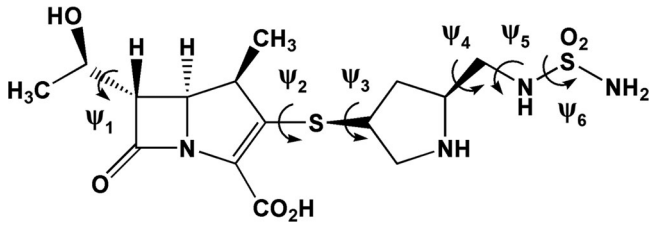

Scheme 1. Schematic drawing of the doripenem molecule. ψ_i's indicate the flexible torsion angles defining the molecular conformation in the solid phases.

or no information at all on its crystal chemistry and on the properties of its (purported) polymorphs could be retrieved[16–19] and, above all, a complete structural analysis is still missing. An EMEA Report[20] briefly states that "The molecular structure of doripenem and the absolute stereochemistry configuration were confirmed by single crystal X-ray crystallography," but we were not able to find, in the scientific or in the forensic literature, any pertinent data, which, therefore, must be kept undisclosed for legal protection issues. Significantly, the existence of a dihydrate form is briefly cited in two patents,[21,22] but the scarce analytical data reported therein, and, above all, the contrasting results proposed for so-called type III and type IV crystals,[21] required a deeper investigation on the subject. Beyond the typical structure–property correlation that a full structural characterization can provide, the detailed knowledge of the crystal structure, in terms of lattice parameters and complete list of fractional atomic coordinates, is absolutely mandatory, if reliable and accurate quantification of a drug within a polycrystalline, or even amorphous, matrix is required (at the product isolation, formulation, and even marketing steps). This is nowadays easily achieved by full-pattern analyses based on the Rietveld method, which with the advent of simple procedures (implemented by diffraction equipment vendors or independently developed—see the QUANTO program[23]) are nowadays deeply pervading the industrial environment.

Thus, based on the complete structure determination of two crystal phases (a monohydrate, Dor*H_2O, the specialty of this drug, and a dihydrate phase, Dor*$2H_2O$), here we report on the rich solid-state chemistry of doripenem, which forms, at a relatively high temperature, a complex anhydrous crystal form (Dor), with two crystallographically independent ($Z' = 2$) molecules in a monoclinic lattice of $P2_1$ space group symmetry. Therefore, this contribution firmly assesses the existence, nature, and stability ranges of several crystalline phases of a widely marketed drug, which suffered of a poor (and cluttered) characterization in the scientific and forensic literature.

METHODS

Origin of the Materials

Doripenem, in its commercially available form (the monohydrated phase), was kindly supplied by ACS Dobfar SpA, (Tribiano, Italy). The dihydrated form, Dor*$2H_2O$, was isolated according to the following procedure: a 5-mL glass vial was charged with finely ground Dor*H_2O (100 mg, 0.228 mmol) and distilled water (100 μL). The mixture was left to evaporate at room temperature for 24 h. The dry white powder was manually ground in an agate mortar and its stability checked by powder diffraction methods, collecting a number of fast diffractometric scans (5°–35° 2θ range, sampling at 0.02° 2θ) over a 24-h period on the instrument described in the following section.

Anhydrous doripenem was prepared *in situ* in the diffractometer cradle using the heating stage described below, by heating Dor*H_2O up to 150°C and maintaining the temperature constant at 150(2)°C during a complete overnight scan.

The chemical and enantiomeric purity of all tested compounds has been controlled by HPLC using C18 and AD-H columns, respectively.

XRPD Analysis

Gently ground powders of the Dor*H_2O and Dor*$2H_2O$ crystal forms were deposited in the hollow of an aluminum sample holder, 0.2 mm deep, equipped with a zero background plate (a quartz monocrystal supplied by The Gem Dugout, State College, Pennsylvania). Diffraction experiments were performed on a vertical-scan Bruker AXS D8 Advance diffractometer in θ:θ mode, equipped with a linear position-sensitive Lynxeye detector, primary beam Soller slits, and Ni-filtered Cu-Kα radiation ($\lambda = 1.5418$ Å). Generator setting: 40 kV, 40 mA. Diffraction data for structure solution of the Dor*H_2O and Dor*$2H_2O$ phases were collected in the 5°–105° 2θ range, sampling at 0.02°, with overall scan time lasting approximately 16 h. Standard peak search methods, followed by profile fitting, allowed the accurate estimate of the low-angle peak position, which, though the SVD indexing algorithm[24] implemented in TOPAS-R (Version 3.0, 2005; Bruker AXS, Karlsruhe, Germany), provided approximate cell parameters: for Dor*H_2O, Monoclinic P, $a = 11.37$, $b = 8.62$, $c = 9.99$ Å, $\beta = 100.37°$, GOF(20) = 28;[25] for Dor*$2H_2O$, Monoclinic P, $a = 13.91$, $b = 8.58$, $c = 8.83$ Å, $\beta = 97.85°$, GOF(20) = 33. Systematic absences indicated $P2_1$ as probable space group for both species, whereas density considerations clearly suggested $Z = 2$.

Structure solutions for Dor*H_2O and Dor*$2H_2O$ were performed by Monte Carlo/Simulated Annealing technique using a rigid model, flexible about the torsion angles $\psi_{1–6}$ and one and two free water molecules, for Dor*H_2O and Dor*$2H_2O$, respectively. H-atoms, to the location and dynamics of which XRPD is substantially blind, were positioned in idealized position—within a protonated COOH tautomer (for the possibility of a zwitterionic form, *vide infra*). Some conformational flexibility was also attributed to the pyrrolidine ring, by freeing torsion angles in a mutually restrained manner. Center of mass location and molecular orientation (five—and not six—extra parameters, $P2_1$ space group allowing arbitrary location of the cell origin along the b-axis) were also determined during the simulated annealing runs. The final refinements were eventually carried out by the Rietveld method, maintaining the rigid bodies introduced at the structure solution stage. The background was modeled by a sixth-order polynomial function of the Chebyshev type; peak profiles were described by the fundamental parameters approach[26] and a common (refinable) isotropic thermal factor was attributed to all atoms. A spherical harmonics description of the lorenztian–peak broadening, caused by anisotropic crystal size effects, was also necessary for the monohydrate phase.[22] The final Rietveld refinements plots for the Dor*H_2O and Dor*$2H_2O$ phases are shown in Figure 1. Fractional atomic coordinates and crystal structure details were deposited with the CCDC (CSD Codes 993835 and