Studies on Solvatomorphism of Betulinic Acid

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ABSTRACT: Five solvates of betulinic acid with dimethyl sulfoxide (I), methanol (II), ethanol (III), isopropyl alcohol (IV), and 2-butanol (V) have been described in this work. Methods of X-ray crystallography, thermal analysis, and Fourier transform infrared spectroscopy were introduced for solvatomorphic identifications and characterizations. The orientation of isopropenyl and carboxylic groups might differ because of single-bonding rotations. The incorporation of solvents resulted in changes of the crystal symmetry, intermolecular arrangements, stoichiometry, hydrogen bonding interactions, and so on. Adducted solvents contributed most to the stability of crystal lattices and led to the formation of crystalline forms. Solvates II–V with their single-crystal structures determined have been reported for the first time. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2696–2703, 2014
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INTRODUCTION

Polymorphs are substances with different unit cells that consist of the same elemental composition.[1] The term solvatomorphism, or pseudopolymorphism, is defined as the ability of a substance to form different unit cells, where these unit cells differ in their elemental compositions through the inclusion of solvent molecules.[2] Different solid-state forms of an active pharmaceutical ingredient can exhibit significant variations of many pharmaceutical-related properties, such as solubility, stability, density, and bioavailability.[3] As for solvatomorphism, although the toxicity of a therapeutic substance may be significantly raised because of the presence of the organic solvent, it can still be of great interest for its researching potentials. Solvates may be the only kind of crystalline forms available to single-crystal X-ray diffraction studies.[4] For example, the only reported crystallographic structures of oleanolic acid and ursolic acid, pentacyclic triterpenoids (PCTTs), in the Cambridge Structural Database (CSD), were both ethanol monosolvates.[5,6] Moreover, solvated forms containing organic solvents were found to be useful intermediates in the purification of paclitaxel.[7] And the dimethylformamide solvate of cefprozil was described to be effective for the preparation as well.[8] Solvates undergoing desolvation may lead to the final products in the pharmaceutical industry.[4] In some cases, even solvated forms of drug substances with exception of hydrates are demonstrated to be final products for clinical use. Two US FDA-approved antitumor drugs, cabazitaxel and trametinib, were in the form of solvates with acetone and dimethyl sulfoxide (DMSO) incorporated, respectively.

Betulinic acid [(3β)-hydroxy-lup-20(29)-en-28-oic acid, $C_{30}H_{48}O_3$, CASRN: 472-15-1] is a naturally occurring plant-derived lupane-type PCTT, which can be isolated from plants in *Betula* spp.,[9] *Diospyros* spp.,[10] *Paeonia* spp.,[11] *Sambucus* spp.,[12] *Syzygium* spp.,[13] *Ziziphus* spp.,[14] and so on. Betulinic acid has been demonstrated to have anti-HIV,[15] antitumor,[16] and anticancer activities,[17] as well as anti-inflammatory,[12] antibacterial,[14] and antimalarial activities.[18] Furthermore, different melting points of betulinic acid have been previously reported (264°C,[14] 282°C,[19] 291°C–292°C,[20] 304°C–305°C,[21] and 316°C–318°C[22]), which may indicate the occurrence of polymorphism or solvatomorphism. Betulinic acid has been demonstrated to exhibit morphological modifications during crystallization via different organic solvents.[23] However, modifications of the crystal habit only reflect the appearance of crystals, which cannot directly refer to changes of crystal forms.[24] Boryczka et al.[25] first reported the crystal structure of betulinic acid DMSO solvate (1:1) in the orthorhombic $P2_12_12_1$ space group, which is now available in the CSD. The unit cell parameters are shown as follows: $a = 6.9417(2)$ Å, $b = 13.8559(5)$ Å, and $c = 31.6232(10)$ Å. However, no more polymorphs or solvates have been found in the literature.

The demonstration of a nonequivalent structure by the single-crystal X-ray diffraction is regarded nowadays to be the most definitive evidence of polymorphism or solvatomorphism. X-ray powder diffraction (XRPD) can also provide unequivocal evidence. Other methods including thermal analysis [e.g., differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA)], infrared (IR) spectroscopy, and melting point determination are helpful for further characterizations.

The chemical structure and reported properties of betulinic acid may indicate the possibility of the existence of different solid forms. This present work describes five crystalline forms of betulinic acid, which were proven to be solvatomorphs with DMSO (I), methanol (II), ethanol (III), isopropyl alcohol (IPA) (IV), and 2-butanol (V). Single-crystal X-ray diffraction, XRPD, DSC, TGA, as well as Fourier transform infrared spectroscopy

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(FTIR) were introduced into the characterizations of these solvates. The incorporation of solvents results in changes of the crystal symmetry, intermolecular arrangements, stoichiometry, hydrogen-bonding interactions, and so on. The crystal structure of solvate I obtained in this study was manifested to be identical with the previously reported one with similar crystallographic parameters. The discovery and characterization of novel solvatomorphs with the most frequently used solvents can offer a better understanding of the structure-activity relationship of betulinic acid in the research and development processes. Crystal structures of solvates II–V have been reported for the first time.

EXPERIMENT

Materials

White powder of betulinic acid raw material was purchased from Wuhan Yuancheng Technology Inc. (batch number: BEA201104211; Hubei, China) and used without further purification. The purity determined by high-performance liquid chromatography was greater than 0.990 mass fractions. All of the solvents used for crystallization were of analytical reagent grade and purchased from Sinopharm Chemical Reagent Company Ltd. (Shanghai, China).

Sample Preparation

Crystalline samples were crystallized by slow evaporation from the respective saturated solutions. About 100 mg of pulverized raw material was added to DMSO–acetonitrile mixture (9:1, v/v), methanol, ethanol, IPA, and 2-butanol, respectively, under constant stirring until saturation was obtained. Solutions were then filtered through filter paper and stored at 10°C over 20 days. Crystalline samples were obtained from crystallization: solvate I, needle shaped, m.p. 292°C–294°C (lit. m.p. 298°C–299°C[25]); solvate II, plate shaped, m.p. 318°C–320°C; solvate III, needle shaped, m.p. 314°C–316°C; solvate IV, plate shaped, m.p. 314°C–316°C; solvate V, needle shaped, m.p. 313°C–315°C.

Single-Crystal X-Ray Diffraction

Colorless single crystals of good quality were preselected microscopically. Single-crystal X-ray diffraction experiments were conducted on a Rigaku MicroMax-002+ diffractometer with a CCD detector, Cu $K\alpha$ radiation (λ = 1.54178 Å) (Rigaku Americas, the Woodlands, Texas). Intensity data of solvates I–V were collected at ambient temperature (293 K). Another dataset measured at low temperature (100 K) was collected for solvate IV. All data were further corrected for absorption and integrated using the CrystalClear software package (Rigaku Americas).

Structures were solved by direct method using SHELXS and refined by full-matrix least-squares calculation against F^2 using SHELXL.[26] Anisotropic displacement parameters (ADPs) were employed for nonhydrogen atoms. Hydrogen atoms were refined isotropically with isotropic atomic displacement parameters (U_{iso}) = 1.2 times the value of the parent atom; hydrogen atoms of methyl or hydroxyl groups were assigned 1.5 times that of the parent atom. Hydrogen atoms were placed in ideal positions and refined using the riding model except for hydrogen atoms involved in the hydrogen bonding, which were detected in the experimental electron density map and refined freely. Refinement of disorders with restraints was introduced to help data convergence.[27]

The volume available to isolated cavities or free solvents in an asymmetric unit of each solvate was calculated by the PLATON program with the probe radius set to be 1.2 Å.[28]

Calculation of X-Ray Powder Diffractometric Patterns

The simulated XRPD patterns were calculated using the MERCURY software (version 3.0; Cambridge Crystallographic Data Center, Cambridge, UK).[29] Detailed conditions were as follows: start angle, 3°; stop angle, 80°; step size, 0.02°.

X-Ray Powder Diffractometric Analysis

Needle-shaped or plate-shaped crystalline samples were grinded in an agate mortar until suitable particle sizes were obtained. XRPD experiments were performed using a Rigaku D/MAX-2550 diffractometer with Cu $K\alpha$ radiation (Rigaku, Tokyo, Japan). Finely pulverized samples were scanned continuously with 2θ coverage of 3°–80° at a constant rate of 8°/min. Data were further processed using the JADE software (Rigaku).

Thermal Analysis

The stoichiometry of solvates was determined by measuring the mass loss when heated in certain temperature range using a thermal gravimetric analyzer. TGA measurements were performed using a Mettler Toledo DSC/TGA 1 calorimeter (Mettler Toledo, Greifensee, Switzerland). Samples were heated from 30°C to 500°C in aluminum oxide cells at a heating rate of 10°C/min under a nitrogen gas flow of 50 mL/min.

A Mettler Toledo DSC 1 calorimeter (Mettler Toledo) was used to mainly investigate thermal events of desolvation in this work. A relatively low temperature range below the melting events of betulinic acid was applied. Samples were heated from 30°C to 230°C in nonhermetically crimped aluminum cells (40 μL) at a constant heating rate of 10°C/min. Both TGA and DSC curves were recorded and further analyzed using the STAR^e software package (Mettler Toledo).

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy measurements were performed using a PerkinElmer Spectrum 400 FTIR spectrophotometer (PerkinElmer, Waltham, Massachusetts). Scanning range was set from 650 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. An attenuated total reflectance sampling accessory was used for measurements. Spectra were recorded and processed using the SPECTRUM software suite (PerkinElmer).

Determinations of Melting Point

Pulverized powder samples were transferred into capillary tubes with one end sealed. Melting points were measured using a Mettler Toledo MP90 apparatus. The rate of temperature rise was regulated to be 3.0°C/min.

RESULTS AND DISCUSSION

X-Ray Crystallography

Crystallographic data and refinement details of solvates I–V are listed in Table 1. The Flack parameter of solvate IV obtained at ambient temperature (293 K) was refined as 0.23(17); the absolute structure could not be properly determined. As a