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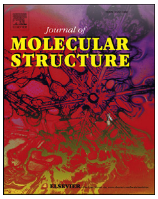

The solid phase of ginkgolide K: Structure and physicochemical properties

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ABSTRACT

Four solvates of ginkgolide K with dimethyl sulfoxide(I), water molecule(II), acetone-isopropyl alcohol(III), methanol-ethanol(IV) and one solvate-free (V) have been described in this work. And the solid-state techniques such as X-ray diffraction, thermal analysis and Fourier transform infrared spectroscopy were used for characterization of the solid phases. The single crystal structures of ginkgolide K solvates (I–IV) have been determined. Ginkgolide K shows strong inflexibility and solvents being incorporated in the crystal structure results in it forming polymorphs via the diverse hydrogen bond interactions.

1. Introduction

The solid forms of active pharmaceutical ingredients (APIs) which may change with the crystallizing conditions include polymorphs, solvates, hydrates etc [1,2]. The physicochemical properties such as melting point, solubility, stability and bioavailability of APIs are related closely to their solid forms [3,4]. Thus, it is remarkably important to understand the solid forms of APIs for the purifying process of APIs, the preparation of bulk drug and the acquirement of final product.

Ginkgolide K (Fig. 1), a natural diterpene compound derived from the leaves of the ginkgo biloba, is found that it not only can strongly inhibit platelet activating factor receptor, but also has powerful neuroprotective and antioxidative properties [5–7]. It could be treated as one of the most promising candidate drugs for treating and preventing cerebral ischemia diseases, drawing the researchers' attention increasingly.

Having been reported in 2001 for the first time, however, there have been no investigations on the solid forms and crystal structures of ginkgolide K till now [8]. Polymorphic form may affect the solubility and the biological activity of ginkgolide K for its poor solubility. Currently, the reports on ginkgolide K mainly focuse on pharmacological activities and pharmacokinetic studies etc [9–11]. Therefore, a screen of polymorph has been performed by slow solvent evaporation and de-solvent by heat in the present study to investigate solid forms of ginkgolide K, and five crystalline forms have been obtained: DMSO (I), hydrate (II), acetone-isopropyl alcohol (III), methanol-ethanol (IV), solvate-free (V), respectively. Moreover, X-ray diffraction, thermal analysis and Fourier transform infrared spectroscopy are used to characterize these solid forms. Furthermore, crystal symmetry, hydrogen bond interactions and the crystal structures of forms I–IV have been analyzed and discussed in this study.

2. Experiment section

2.1. Materials

Ginkgolide K raw material is white crystalline power which is provided by Jangsu Kanion Parmaceutical Co.,Ltd. (Jiangsu, China). Its purity measured by HPLC is more than 98%. All the solvents used for crystallization are of analytical reagent grade and obtained from Sinopharm Chemical Reagent Company Ltd. (Shanghai, China).

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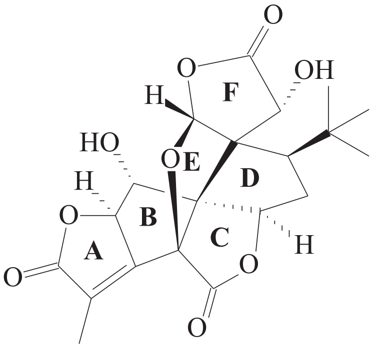

Fig. 1. Structure of ginkgolide K.

2.2. Sample preparation

Five crystalline forms have been acquired in this research, with forms I–IV harvested by slow evaporation of solvent. Approximately 100 mg of Ginkgolide K was completely dissolved in DMSO-water mixture (2:1, v/v), water, acetone-isopropyl alcohol mixture (2:1, v/v) and methanol-ethanol mixture (1:1, v/v) at 40 °C, respectively. Then, the obtained solutions were slowly cooled and allowed to evaporate at ambient condition. Finally, four single crystals of ginkgolide K suitable for SXRD experiments were obtained: DMSO (I), hydrate (II), acetone-isopropyl alcohol (III), methanol-ethanol (IV), respectively. The samples obtained from methanol-ethanol mixture always existed in the impure crystalline phase, thus, only Single-crystal X-ray diffraction (SXRD) was applied to characterize the sample of form IV. In addition, form V was prepared by heat of form II at 150 °C for 20 min.

2.3. Single-crystal X-Ray diffraction (SXRD)

SXRD experiments were performed on a Rigaku MicroMax-002 + diffractometer with a CCD detector, *Cu K* radiation ($\lambda = 1.5417$ Å) (Rigaku Americas, the Woodlands, Texas). Intensity data of forms I–IV were collected at ambient temperature (293 K). 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 **[12]**.

Hydrogen atoms were refined isotropically with isotropic atomic displacement parameters (Uiso) = 1.2 times the value of the parent atom, placed in ideal positions and refined using the riding model except for hydrogen atoms bonded to oxygen atoms, which were determined on the experimental electron density map.

2.4. X-ray powder diffraction (XRPD)

Crystalline samples were grinded fully in an agate mortar. XRPD experiments were performed on a Rigaku D/MAX-2550 diffractometer using *Cu K* radiation (Rigaku, Tokyo, Japan). Samples were scanned continuously at a constant rate of 8°/min in the range of 3°–80°. Data were further processed using the JADE software (Rigaku).

2.5. Thermal analysis

The stoichiometry of solvates was determined by measuring the mass loss of them heated in certain temperature range using a thermal gravimetric analyzer (TGA). TGA measurements were performed on 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 investigate thermal events of desolvation in this work. Samples were heated from 30 °C to 300 °C in sealed aluminum cells (40 uL) at a constantly heating rate of 10 °C/min. STAR^{e} software package (Mettler Toledo) were employed to record and further analyze the data.

2.6. 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 by the SPECTRUM software suite (PerkinElmer).

3. Results and discussion

3.1. X-ray crystallography

The skeleton of Ginkgolide K consists of six rings (A–B–C–D–E–F) which shows a plane–envelope–plane–envelope–envelope-envelope conformation, and adjacent rings all adopt cis-connection. Additionally, ginkgolide K contains 8 chiral centers. The four obtained crystals (form I–IV) in the present study crystallized in the first space group, with the Flack parameters:0.03(2), 0.17(8), −0.09(13), 0.09(16), therefore, the absolute structures could be determined on the Flack parameters **[13]**. The crystallographic parameters of forms I–IV are listed in Table 1. The compound has strong inflexibility, and the similar conformations are observed in the form I–IV (Fig. 2). The various polymorphic forms of Ginkgolide K result from the adduction of diverse solvates involved in the formation of different hydrogen bonds. The disorder phenomenon is only observed in the solvent molecules due to the strong inflexibility of ginkgolide K, which could account for the molecules of DMSO in solvate I, isopropyl alcohol in solvate III and the ethanol in solvate IV, dealt with occupying two positions, showing disorder. The four crystalline forms of Ginkgolide K all crystallize in orthorhombic crystal system. Form I–III all belong to $P2_12_12_1$ space group, whereas form IV belongs to $P22_12_1$ pace group.

The crystalline forms of Ginkgolide K all contain different solvent molecules in the present study. The calculated volumes of solvent-accessible voids for forms I–IV are 673.1(27.9%), 57.9(2.9%), 1707.3(33.6%), 791.2(31.4%), respectively, by PLATON program. The minimal solvent-accessible void for form II suggests that the molecules in it arrange themselves more closely than other solvates, with the biggest density of 1.426.

There are two hydroxyls (O6 and O9) as hydrogen bond donors and three carbonyls (O5, O7 and O8) as hydrogen bond acceptors in ginkgolide K, which easily let it interact with solvents to form hydrogen bond in the crystallizing process. The interaction way of hydrogen bond between solvent molecules and ginkgolide K varied from the involved solvents, and the detail hydrogen bond geometric parameters are summarized in Table 2. The hydrogen bond interactions of forms I–IV are present in Fig. 3. In brief, both hydroxyls of forms I– IV is involved in hydrogen bonds. It could be observed that all hydroxyls O9 are bonded to solvent molecule via intramolecular hydrogen bonds among forms I–IV. While hydroxyls O6 of forms I–IV could be grouped into two categories. In the first category, hydroxyl O6 of form I bridges the O7 (-x, y+1/2, -z-1/2) via intermolecular hydrogen bond. In the second category,