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Physical characterization of oleanolic acid nonsolvate and solvates prepared by solvent recrystallization

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Abstract

Oleanolic acid is a naturally occurring compound used clinically in China for the treatment of hepatitis B. The solid-state chemistry of oleanolic acid recrystallized from a variety of solvents was investigated. Glassy materials were prepared from dichloromethane and chloroform solvents. The oleanolic acid non-solvate prepared from acetone (OA-acetone), and the two oleanolic acid solvates prepared from methanol (OA-methanol) and ethanol (OA-ethanol) were physicochemically characterized. Upon desolvation, both the methanol and ethanol solvates were found to undergo phase transformation to a crystalline phase similar to OA-acetone around 190–195 °C. The PXRD patterns of commercial pharmaceutical grade OA and the OA-methanol were similar, so the commercial form is probably desolvated oleanolic acid methanol solvate.

Keywords: Oleanolic acid; Solvates; Solvent recrystallization; Solid-state transition

1. Introduction

Oleanolic acid (OA), a naturally occurring pentacyclic triterpenoid (Fig. 1a), is a biologically active marker compound commonly present in Chinese herbs such as *Akebia trifoliata* (in Chinese, *mu tong*) and listed in the 2005 China Pharmacopoeia. Pharmacological studies of OA have reported that it offers hepatoprotection against chemically-induced liver injury (Liu, 2005). The underlying mechanisms are possibly related to anti-oxidant activity, anti-inflammatory action, and its induction of metallothionein, a small cysteine-rich protein acting like glutathione in the body's defense against toxic insults (Liu, 2005). In addition, it has been recently demonstrated that OA's hepatoprotective action works at least in part through inhibiting the liver's mitochondrial permeability transition (Tang et al., 2005). OA in tablet form is available in China over the counter as a health supplement. Clinically, it has been used in the treatment of hepatitis, and as an adjunct therapy to prevent hepatotoxicity induced by anti-tuberculous medications such as isoniazid and pyrazinamide (Wang, 2003; Chen et al., 2005).

Being hydrophobic (Fig. 1a), OA exhibits solubility problems. Pharmaceutical grade OA does not dissolve to a detectable level (<1 μg/ml by HPLC) in buffer solutions (at pH 1 or 7) after 120 min at 37 °C (unpublished data). Due to poor absorption and extensive metabolic clearance, OA has an absolute bioavailability in rats of only 0.7% at oral doses of 25 and 50 mg/kg (Jeong et al., 2007). In a human pharmacokinetic study of OA capsules, T_{max} was reported to be 5.2 h, indicating that OA is not released immediately (Song et al., 2006). The potential therapeutic benefits of OA are widely known, but there has not been any detailed investigation of the solid-state properties which may account for its poor aqueous solubility and oral absorption. The present study aimed to prepare different crystalline forms of OA by solvent recrystallization and to characterize them using established solid-state techniques. The objective was to gain a better understanding of the solid-state behavior of OA which might enable the development of a more consistent and efficacious OA oral formulation.

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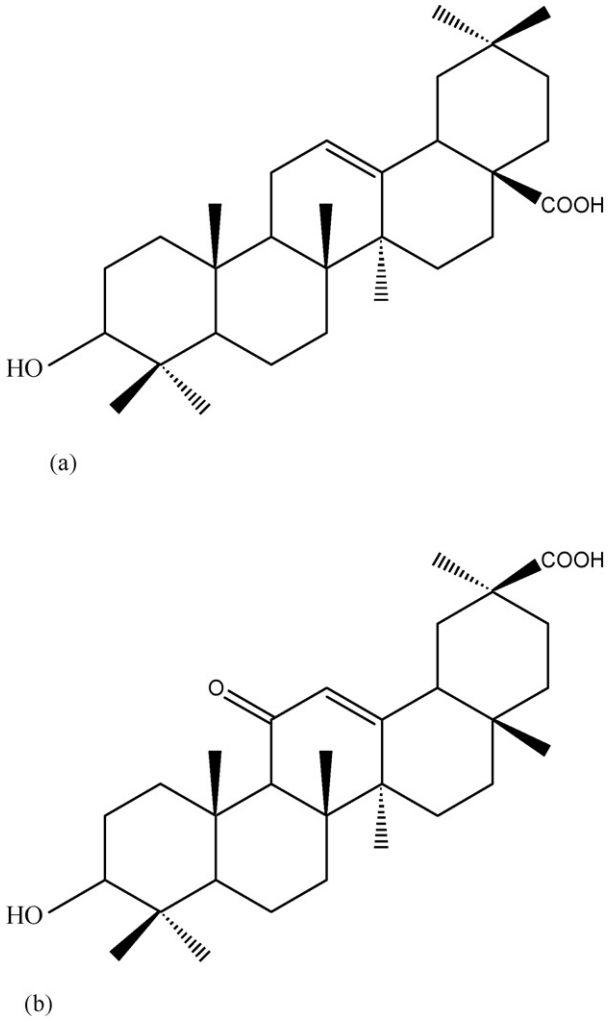

Fig. 1. Chemical structure of (a) oleanolic acid; (b) glycyrrhetinic acid.

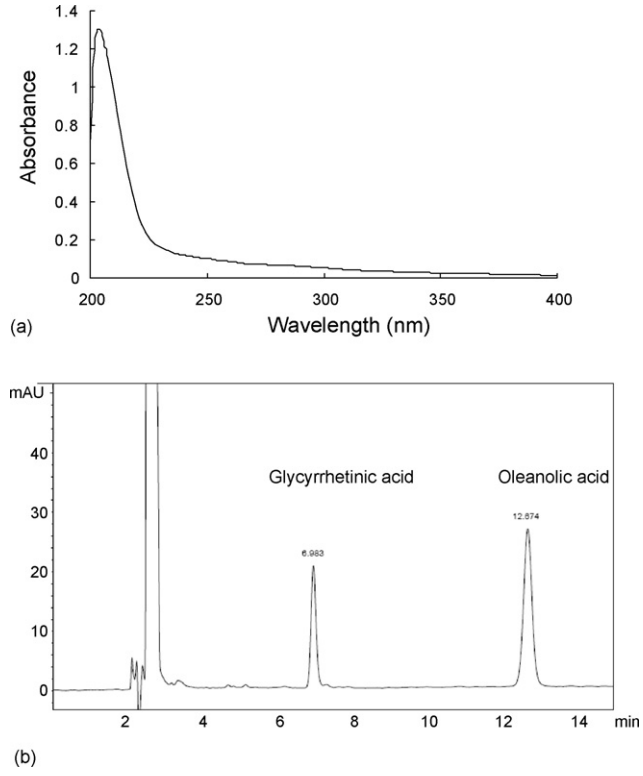

Fig. 2. (a) UV spectrum of oleanolic acid in absolute ethanol; (b) HPLC chromatogram in oleanolic acid assay.

2. Materials and methods

2.1. Materials

Pharmaceutical grade OA raw material (OA–RW) (purity ≥ 95%) was purchased from International Laboratory, San Bruno, USA. Standard OA (minimum purity 97%) was purchased from Sigma, USA, and a glycyrrhetinic acid standard was obtained from the China's National Institute for the Control of Pharmaceutical and Biological Products. The standards were used without further purification. All the methanol, absolute ethanol, dichloromethane, chloroform, acetonitrile and acetone used were of either analytical or HPLC grade. Sodium dodecyl sulphate was purchased from USB Corporation, USA. All the water used was double distilled.

2.2. Preparation of OA through solvent recrystallization

OA samples were prepared using cold crystallization or solvent evaporation methods. In the cold crystallization method, OA–RW powder was dissolved in an appropriate organic solvent at a temperature close to the boiling point of the solvent, and the solution was then cooled within a few hours down to 4 °C. In the solvent evaporation method, OA–RW was dissolved in an appropriate organic solvent at room temperature, and the solution was then allowed to evaporate at room temperature in a dark place for several weeks until visual examination showed that it was dry. In both methods, the OA was then harvested by filtration and dried on the filter paper with minimal trituration.

2.3. UV and HPLC characterization of oleanolic acid

The UV spectrum of oleanolic acid in absolute ethanol at 60 μg/ml was recorded using a DU-640 UV/vis spectrometer from Beckman Coulter, USA (Fig. 2a).The HPLC analysis of the oleanolic acid followed the protocol reported by Chen et al. (2003), with slight modifications. The analysis employed an Agilent 1100 series HPLC system, a 250 mm × 4.6 mm Agilent 5 μm Zorbax SB-C18 column, and a photodiode array detector (DAD) scanning the 190–400 nm range. Injection volume was 20 μl. The mobile phase was composed of acetonitrile and 0.5% phosphoric acid (85:15). It was eluted isocratically at a flow rate of 1.0 ml min^{-1}. The wavelength chosen for oleanolic acid quantification was 204 nm. Glycyrrhetinic acid, with a chemical structure similar to oleanolic acid (Fig. 1b), was used as an internal standard at 15–32 μg/ml. The glycyrrhetinic acid and oleanolic acid eluted as two distinct peaks with retention times of 6.9 min and 12.7 min, respectively (Fig. 2b). A calibration curve based on the AUC ratio between oleanolic acid and glycyrrhetinic acid had excellent linearity ($R^2 > 0.9994$). The