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Conversion of steroid saponins into diosgenin by catalytic hydrolysis using acid-functionalized ionic liquid under microwave irradiation

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ABSTRACT

The catalytic hydrolysis with an acid-functionalized ionic liquid under microwave irradiation was successfully developed to convert steroid saponins into diosgenin. The typical acid-functionalized ionic liquid, 1-sulfobutyl-3-methylimidazolium hydrosulfate ($[BHSO_3MIm]HSO_4$), was used to evaluate the catalytic efficiency. The results strongly suggested that acid-functionalized ionic liquid under microwave irradiation significantly improved the hydrolysis efficiency of steroid saponins. In addition, we optimized hydrolysis parameters, including the ionic liquid concentration, the ratio of solvent to solid, the reaction temperature, and reaction time. Under the optimal conditions, this approach achieved the highest yield of diosgenin (29.97 ± 0.51 mg) with 0.3 g steroid saponins. Compared with regular hydrochloric acid hydrolysis, the developed approach obtained 96% diosgenin and reduced 93% reaction time, indicating that the catalytic hydrolysis with an acid-functionalized ionic liquid under microwave irradiation had a broad application prospect in diosgenin production.

1. Introduction

The medicinal plant *Dioscorea zingiberensis* C. H. Wright (DZW), which belongs to dioscoraceae family, is widely distributed in Shanxi, Hubei, and Yunnan Provinces in China (Zhang et al., 2012). Its rhizome is a well-known Traditional Chinese Medicine. And it is the main source of diosgenin (Wang et al., 2008), a starting material for the semi-synthesis of drugs of steroidal hormones, such as oral contraceptives, sex hormones, and other steroids (Oncina et al., 2000). In recent years, various medicinal usages of diosgenin, including anti-thrombosis effect (Gong et al., 2011), anti-oxidation (Rajalingam et al., 2012), anti-proliferation, and anti-invasion effects on cancer cells (Mao et al., 2012), have been reported.

In the plants of *Dioscorea Linn.*, diosgenin is stored in the form of saponins, which are regarded as the major active components of DZW (Liu et al., 2010a, b, c). Saponins are connected with glucoses or rhamnoses to aglycone with C–O glucosidic bonds (Zhang et al., 2006). Industrial diosgenin is mainly prepared through hydrochloric acid hydrolysis of these glucosidic bonds. However, a large quantity of wastewater with low pH and the high level of chemical oxygen demand (COD) is discharged during the industrial production of diosgenin, thus leading to serious environmental pollution. Moreover, secondary reactions, such as chlorinated reaction, in inorganic acid hydrolysis decrease the production of diosgenin.

In order to reduce environmental pollution, many hydrolysis technologies for the conversion of steroid saponins to diosgenin have been studied to replace inorganic acid hydrolysis technology. *Trichoderma reesei* (Zhu et al., 2010), *Trichoderma harzianum* (Liu et al., 2010a, b, c), and *Aspergillus oryzae* (Dong et al., 2010) could produce diosgenin with less pollution. Microorganisms synthesize a series of glycosidases to hydrolyze steroid saponins. The direct microbial transformation method of plant materials has been widely employed due to its low cost and comprehensive utilization way of starch in the plant. Starch is not hydrolyzed under acid conditions, but it can be fully utilized by microorganisms under acid conditions. It greatly reduces the emissions of high levels of COD. However, multi-substrates and enzymes in raw herbs during biotransformation were not thoroughly investigated and a high conversion rate was not obtained (Dong et al., 2010). Furthermore,

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biotransformation is non-directional conversion and many intermediate products are produced by microorganisms during biotransformation. A large quantity of organic solvent is consumed during separation and purification of target components. Compared with the traditional method, the biotransformation method usually has the long production cycle of 3–7 days and the low production efficiency. Therefore, many researchers paid their attentions to enzymatic hydrolysis. Enzymatic hydrolysis from saponins to diosgenin was carried out by β-glucosidase from *Aspergillus fumigates* (Lei et al., 2012) or commercial cellulose (Liu et al., 2010a, b, c). Enzymatic hydrolysis of natural products is an effective method and has the following advantages: high specificity, mild reaction conditions, and environment-friendly process. However, this method requires the preparation process of enzymes. However, the low catalytic activity for specific glycosidic bond leads to the low yield. Moreover, this method cannot be applied in industrial production because of its high cost.

Therefore, it is necessary to develop an efficient, convenient, and economical conversion method. In recent years, ionic liquids, as a kind of environment-friendly solvent, have shown great potential in replacing conventional organic solvents in many fields (Wu et al., 2013; Naushad et al., 2012; Yu et al., 2011; Yi et al., 2013). Ionic liquids are composed of various cations and anions and ionic liquids at room temperature are still in the liquid state. Therefore, they are non-volatile and non-flammable and can be miscible with water and various organic solvents (Wilkes, 2004; Boschetti et al., 2007). Ionic liquids show the outstanding design capability compared with conventional solvent systems. Moreover, ionic liquids are recyclable and environmentally compatible and can alleviate environmental pollution. Therefore, we adopted ionic liquid as a "green catalyst" in the hydrolysis reaction. Acid-functionalized ionic liquid shows the excellent catalytic hydrolysis performance and has the characteristics of conventional ionic liquids. In addition, due to the introduction of acid functional groups, it has some other peculiarities, such as uniform acid intensity distribution, high acid density, adjustable acidity, and durable acidity.

In order to shorten reaction time, we used microwave heating method instead of conventional thermal methods. Microwave has attracted considerable attentions because its heating effect can greatly shorten reaction time and significantly improve the rate and yield of reactions (Huang et al., 2006). Ionic liquids are composed of a pair of ions and therefore have a high density of strong dipoles, which make them promising candidates for microwave absorption (Shih et al., 2011).

To the best of our knowledge, acid-functionalized ionic liquid catalytic hydrolysis of steroid saponins under microwave irradiation was not reported. In this study, we combined ionic liquids with microwave technologies to achieve the efficient and clean production of diosgenin (Fig. 1). In addition, important reaction factors, such as ionic liquid concentration, the ratio of solvent to solid, and hydrolysis time, were studied. The recovery method was also investigated. All solvents used in hydrolysis are environment-friendly and recyclable. Finally, this method was compared with the traditional method to highlight its advantages.

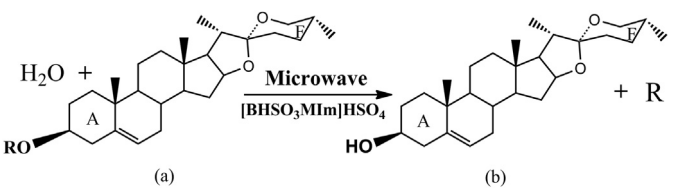

Fig. 1. Diosgenin production by acid-functionalized ionic liquid $[BHSO_3MIm]HSO_4$ under microwave irradiation. (a): Steroidal saponins. (b): Diosgenin. R: A number of sugars, such as glucoses and rhamnoses.

2. Experimental

2.1. Materials

D. zingiberensis C. H. Wright rhizomes were dried at 50 °C and then crushed into powder. The preparation method of total saponins was based on the report by Yang et al. (2003). Dry rhizome powder was mixed with 70% aqueous EtOH and heat reflux extraction was carried out twice at 60 °C. Then the extraction solution was filtered, and concentrated in the rotary evaporator to recover ethanol. The rest aqueous solution was partitioned with *n*-BuOH for three times to obtain saponin extracts. Then *n*-BuOH was recovered and total steroid saponins were obtained.

Acid-functionalized ionic liquid 1-sulfobutyl-3-methylimidazolium hydrosulfate ($[BHSO_3MIm]HSO_4$, purity > 99%) was purchased from Lanzhou Institute of Chemical Physics (Gansu). Diosgenin standard was purchased from Shanghai Yuanye Biological Technology Co., Ltd. (Shanghai). The purity of standard compound was higher than 99%.

Chromatographic grade of acetonitrile was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai). All the solvents prepared for HPLC (high-performance liquid chromatography) were filtered through the 0.22-μm microporous membrane.

Petroleum ether and *n*-BuOH were all of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai).

2.2. Apparatus

XH-800B intelligent microwave workstation with an output power of 0–1000 W was purchased from Beijing Xianghu Science and Technology Development Co., Ltd. (Beijing). The temperature of the reaction mixture was monitored and kept constant (±1 °C). A positive & negative controlled rotary plate was placed on the floor of the microwave cavity and six samples could be processed at the same time. In the actual experiments, the reaction temperature was kept constant through fluctuant microwave power.

The Agilent 1100 series HPLC system was purchased from Agilent (California, USA). A GraceSmart RP C18 column (5 μm, 4.6 × 250 mm. W. R. Grace & Co.-Conn, Columbia, Maryland, USA) was used.

2.3. Optimization of microwave-assisted hydrolysis

In order to get the optimal hydrolysis, four experimental parameters, the concentration of ionic liquid, the ratio of solvent to solid, the reaction temperature, and reaction time, were investigated and optimized by univariate analysis method. In the experiments, ionic liquid $[BHSO_3MIm]HSO_4$ was fully dissolved in deionized water in a beaker, transferred to a volumetric flask, and diluted to different concentrations. Then 0.3 g steroid saponins were weighed, added into a reaction tank, and then mixed with a certain volume of ionic liquid. Reactions were carried out in a microwave cavity by the pre-set program with different parameters. The instrument automatically started the cooling process immediately after the reaction was completed. After cooling, the solution was filtered and washed with distilled water for several times. The filtrated residue was dried at 50 °C, and then extracted in a soxhlet extractor for 3 h with petroleum ether. The solvent extracts were concentrated and dried in a rotary evaporator. Then diosgenin was dissolved with methanol and then filtered through the 0.22-μm membrane. Diosgenin was determined by HPLC.

2.4. Recycling experiment

Ionic liquids are recyclable. Therefore, ionic liquid recovery method was also studied. After each reaction, reaction products,

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