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The mechanism of hydrothermal hydrolysis for glycyrrhizic acid into glycyrrhetinic acid and glycyrrhetinic acid 3-*O*-mono-β-D-glucuronide in subcritical water

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ABSTRACT

To improve the bioactivity and sweetness properties of glycyrrhizic acid (GL), the hydrothermal hydrolysis of GL into glycyrrhetinic acid (GA) and glycyrrhetinic acid 3-*O*-mono-β-D-glucuronide (GAMG) in subcritical water was investigated. The effects of temperature, time and their interaction on the conversion ratios were analyzed and the reactions were elaborated with kinetics and thermodynamics. The results showed that GL hydrothermal hydrolysis was significantly ($P < 0.05$) affected by reaction time and temperature, as well as their interaction, and could be fitted into first-order kinetics. The thermodynamic analysis indicated that the hydrolysis of GL was endergonic and non-spontaneous. The hydrolytic pathways were composed of complex consecutive and parallel reactions. It was concluded that subcritical water may be a potential medium for producing GAMG and GA.

1. Introduction

Liquorice has been widely used in Chinese traditional medicine to cure phthisis, contagious hepatitis and bronchitis based on its anti-inflammatory, antiviral and antineoplastic properties (Wang & Yang, 2007). There are more than 400 compounds separated from different Glycyrrhiza species, and triterpene saponins are proven to be the major component (Cinatl et al., 2003).

Glycyrrhizic acid (GL), composed of one molecule of aglycone with two molecules of glucuronic acid (Fig. 1a), is a natural sweetener in liquorice (Cinatl et al., 2003) and has been widely used in the tobacco, food and pharmaceutical industries (Wang et al., 2012). GL also possesses many useful pharmacological activities, such as anti-cancer, anti-inflammatory and anti-bacterial properties (Zhang & Ye, 2009). However, as a bioactive compound, GL is not an optimal molecule for absorption in the bloodstream and disturbs ionic metabolic equilibrium in many organisms, which can cause hypertension (Cinatl et al., 2003).

Glycyrrhetinic acid (GA), the aglycon of GL, is produced by GL hydrolysis, which removes two molecules of glucuronic acid moieties. GA is a component of liquorice, which can be completely hydrolyzed by intestinal bacteria and enter into the body's systematic circulation (Krähenbühl, Hasler, & Krapf, 1994). Both GL and GA can inhibit the proliferation of hepatoma carcinoma cells, with the dose of GA only 2.5% of GL to obtain an equal effect (Zhang & Ye, 2009). In addition, GA has a more significant effect on *in vitro* anti-platelet aggregation compared to GL. GA also exhibits cytotoxicity against tumor cells as well as inhibitory activities on rotavirus infections (Kim et al., 2000). Therefore, GA has some advantages over GL (Farese et al., 2009), and is considered as a promising lead compound for designing a more efficacious and less toxic chemosensitizing agent to enhance the efficacy of cancer chemotherapy (Maatooq, Marzouk, Gray, & Rosazza, 2010).

Glycyrrhetinic acid 3-*O*-mono-β-D-glucuronide (GAMG) is formed after cleaving the distal glycosidic bond of GL (Fig. 1a), and has a higher bioactivity, stronger physiological function, more pleasant sweetness and a lower caloric value compared to those of GL (Ohtake et al., 2007). As a sweetener, its sweetness intensity is 5-fold higher than that of GL, and GAMG is much safer as its

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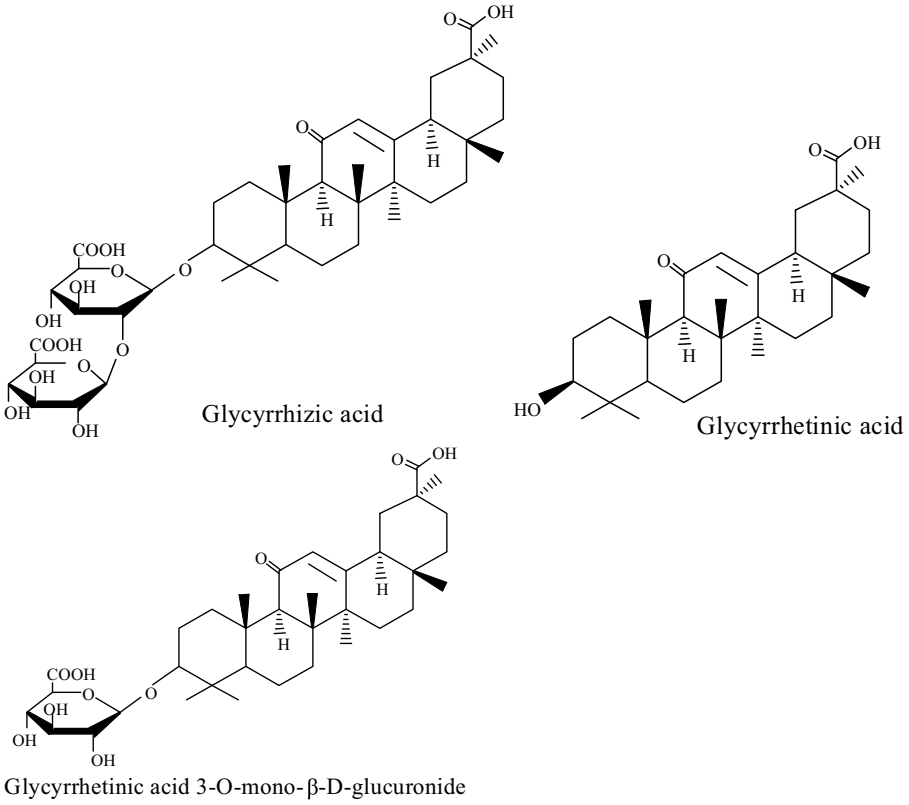
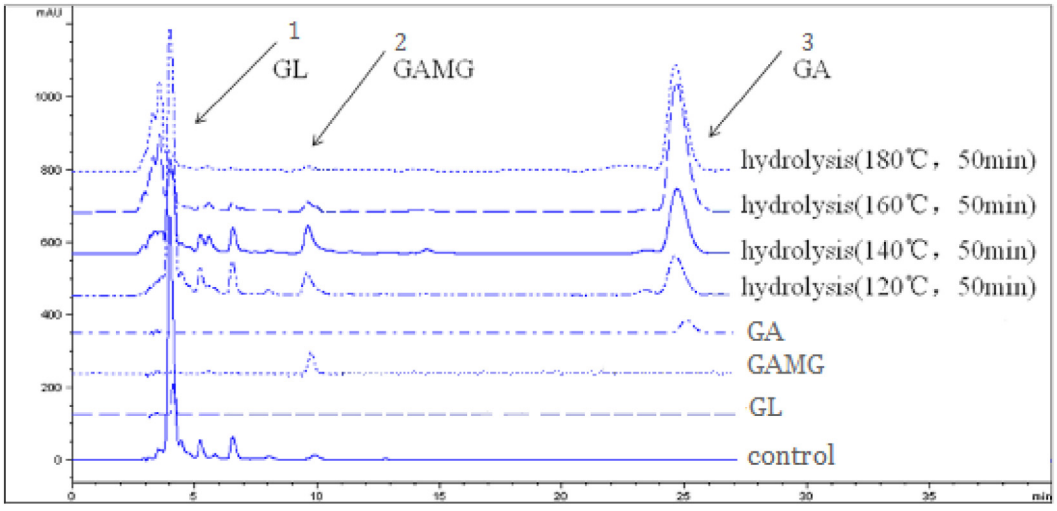


(a)



(b)

Fig. 1. HPLC profiles of compounds in hydrolysate of GL, (a) the structures of glycyrrhizic acid (GL), glycyrrhetinic acid (GA) and glycyrrhetinic acid 3-*O*-mono-β-D-glucuronide (GAMG); (b) the HPLC profiles before and after hydrolysis of GL.

median lethal dose (MLD_{50}) is 5 g/kg bw, while the MLD_{50} of GL is 0.8 g/kg bw (Feng, Li, Xu, & Wang, 2006). There are many similar characteristics between GAMG and GL in drug formulation, such as antivirus, anti-inflammatory and anti-tumor properties, however, the biological availability of GAMG is higher than that of GL (Ohtake et al., 2007). Therefore, GAMG is being considered as a promising and excellent food sweetener and therapeutic agent.

GA and GAMG are traditionally hydrolyzed from GL with hot concentrated mineral acid over 10 h (Kim, Lee, & Han, 1999). GA and GAMG were also reported to be produced from GL hydrolysis with β-D-glucuronidase (Huang et al., 2009). However, the process of β-D-glucuronidase biocatalysis was difficult to realize in industrial-scale production because of its high cost, low activity and biocatalyst selectivity (He et al., 2010). In addition, the large-scale production of GAMG was limited because of the poor hydrophilicity of both GL and GAMG (Chen, Kaleem, He, Liu, & Li, 2012). It was reported that the fungus *Penicillium purpurogenum* Li-3 could produce specific β-D-glucuronidase, which possessed high chemical bond selectivity and could be directly used for producing GAMG. However, the yield of specific β-D-glucuronidase was relatively low (Feng et al., 2006). There is another method with high yield of β-D-glucuronidase, the gene pgus (GenBank Accession