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Structural stability of acetyl saponins in different solvents and separation materials

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ABSTRACT

Two acetyl saponins, 2″-O-acetylplatycodin D and 3″-O-acetylplatycodin D from *Platycodon Radix* were selected for their structure stability study. Different solvents, stationary phases and temperatures were employed to study the structural inter-conversion of acetyl group in two acetyl saponins. The results showed that the reaction of acetyl transfer was faster in water than other solvents, and comparing to the normal/reverse silica gels, the reaction of acetyl migration almost did not happen during the process of purification by macroporous resin. The activation energy and enthalpy of 2″-APD converted into 3″-APD reaction were 63.01 kJ mol^{-1}, and 7.48 kJ mol^{-1}, respectively. Low polar solvent, macroporous resin and low temperature may be more suitable for the separation and purification of acetyl saponins.

1. Introduction

In the process of separation and purification for natural products, the active ingredients such as polyphenols and acetyl saponins often undergo chemical structure change, which may make the purification difficult (Cheynier, 2005; Crublet et al., 2002; Guo and Kenne, 2000). Various separation conditions may significantly affect the chemical structure stability of nature-original chemicals (Jacobsen et al., 1996). A more comprehensive understanding of the transformation of the unstable compounds is necessary, which helps to avoid obtaining misleading results of nature products research.

The acetyl saponins are typical unstable compounds, which are extensively distributed in many species of medicinal herbs (Sparg et al., 2004). However, owning to the low activation energy of acetyl migration reaction, it may be difficult to obtain the pure acetyl saponin, which may restrict further research of quality control and medicinal application. In this study, two acetyl triterpenoid saponins named 2″-O-acetylplatycodin D (2″-APD) and 3″-O-acetylplatycodin D (3″-APD) from *Platycodi Radix* were selected to study the chemical structure stability (Fig. 1). Different solvents, stationary phases and temperatures were used to study the effects on the structural inter-conversion of acetyl group in two acetyl saponins.

2. Results and discussion

From the data of Table 1, normal phase silica gel had the greatest effect on structure stability of the acetyl saponins. Comparing to other stationary phases, as much as 42% of 2″-O-acetylplatycodin D was converted into 3″-O-acetylplatycodin D (Fig. 1) using normal phase silica gel as stationary phases. The pure acetyl saponin eluted from macroporous resin nearly did not undergo any structure transformation. The determination results of structural inter-conversion of the two compounds were shown in Table 1 using HPLC.

The separation mechanism of silica gel is based on a combined effect of adsorption and distribution, which is mainly attributed to the silanol groups distributed on the surface of silica gel (Bldiingmeyer et al., 1982). However, the silanol, a polar group with weak acidity, may dissociate H^+, which possibly conduct an electrophilic attack on carbonyl oxygen of the acetyl group, and catalyzes the acetyl group to transfer from one hydroxyl of rhamnose to another. The possible acetyl migration mechanism was shown in Fig. 2.

Solvent also showed influence on structural stability of the acetyl saponin. Different percentages of 2″-APD converted into 3″-APD in three solvents (Fig. 3), and the transformation occurred faster in water than other solvent, which indicated that water might not be suitable as solvent for extraction and isolation of the

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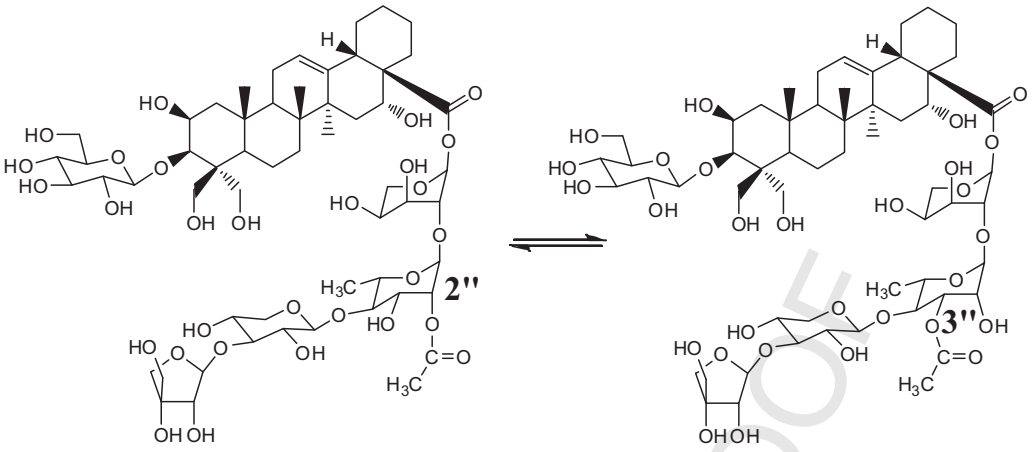

Fig. 1. Structures and transfer reaction of 2″-O-acetylplatycodin D and 2″-O-acetylplatycodin D.

Table 1
Structural inter-conversion of the two acetyl saponins in different separation materials.

Initial compound	Inter-conversion	Proportion of the two acetyl compounds		
		Normal phase silica gel	Reverse phase silica gel	Macroporous resin
2″-APD	2″-APD → 3″-APD	1:0.73	1:0.52	1:0.01
3″-APD	3″-APD → 2″-APD	1:0.94	1:0.37	1:0.02

2″-APD, 2″-O-acetylplatycodin D; 3″-APD, 3″-O-acetylplatycodin D.

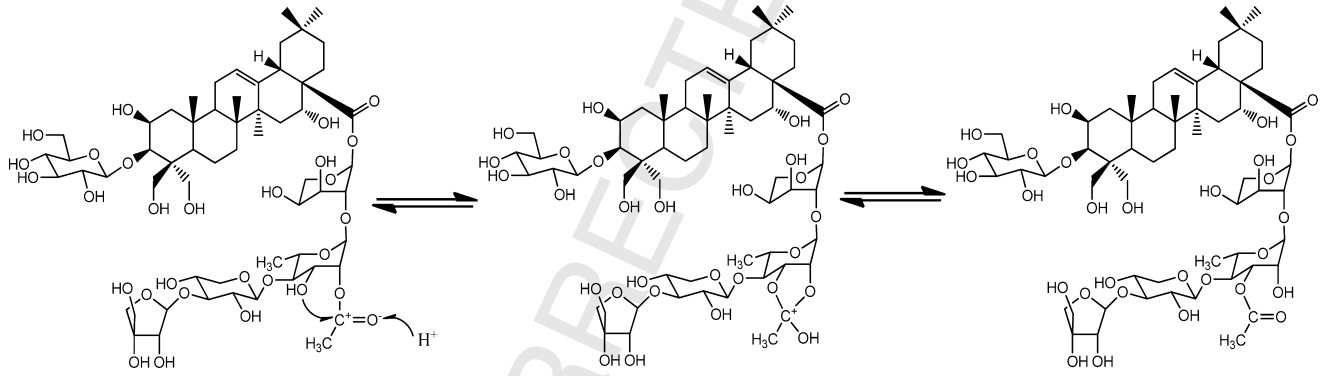

Fig. 2. Possible mechanism of acetyl group migration.

acetyl saponin. In addition, there would be much foam when saponins dissolved in water, which also made separation difficult. Although frequently used as mobile phase in analytical or preparative HPLC separations of saponin (Ha et al., 2006), it was also found that the acetonitrile–water solvent could cause the acetyl group migration between the two compounds. However, it should be noted that when sample containing both 2″-APD and 3″-APD loaded onto column the acetyl transfer reaction did not happen. The results indicated that a more polar solvent made the acetyl group transferred faster. Most solvent systems used to separate acetyl compounds from *Platycodi Radix* were less polar, and the separation method was mainly high speed counter current chromatography (HSCCC), which had no silica gel-based stationary phase (Skalicka-Woźniak and Garrard, 2014; Ha et al., 2011; Ishii et al., 1984). Though this method might avoid problem of structural inter-conversion of the acetyl saponins, it would take much time to find a proper solvent system. Our study provided a simple alternative procedure based on macroporous resin as separation material to obtain pure unstable compounds.

The effect of temperature on the transfer reaction was significant. When temperature was above 40 °C, the reaction rate was too fast to monitor the concentration change of the saponins with HPLC. The temperature was set up at a range of 0–35 °C. A typical HPLC chromatogram was obtained by continuously injecting sample solution of transfer reaction at certain time intervals and shown in Fig. 4. From HPLC chromatogram, the changes of peak area of 2″-APD or 3″-APD related to the time were observed and the rate constants and the equilibrium constants of transfer reaction (Fig. 1) were obtained at different temperatures and listed in Table 2. The rate constants and equilibrium constants of the transfer reaction both increased with increase of temperature. The activation energy of 2″-APD to 3″-APD and 3″-APD to 2″-APD were calculated as 63.01 kJ mol^{-1} and 55.52 kJ mol^{-1}, using the rate constants at different temperatures. The results indicated that acetyl group was more inclined to transfer to 2″-OH on rhamnose at room temperature, which complied with the determination results of saponins from this plant (Yoo et al., 2011). The enthalpy of reaction of 2″-APD converted into 3″-APD

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