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An efficient, scalable approach to hydrolyze flavonoid glucuronides via activation of glycoside bond

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ABSTRACT

Hydrolyzing flavonoid glucuronides into corresponding aglycones posed some significant challenges. To improve acid-catalyzed hydrolysis process of flavonoid glucuronide, structures of glucuronide, hydrolysis parameters and post-processing were optimized. The optimized condition was performed by hydrolysis flavonoid glycoside methyl ester in a mixed solvent consisting of 2 mol/L H_2SO_4/EtOH/H_2O (1/8/1, v/v/v) at 95 °C for 7 h and resulted in up to 90% aglycone yields, minimal byproduct formations and milder hydrolysis conditions. Furthermore, the optimized method avoids tedious purification steps and is easily conducted on a relatively large-scale using economical and commercially available reagents.

1. Introduction

Flavonoids, a broad class of polyphenolic secondary metabolites from natural source, consist of flavonoid aglycones (FA) and flavonoid glycosides (FG). Variation in the molecular structures of FG originates from the different type of aglycone, sugar and the number of sugar groups, among which the fraction of glucuronide is quite common.[1] Flavonoids have gained much attention from researchers and the food industry due to the fact that they are natural antioxidants as an alternative to synthetic products for the usage in food and cosmetics,[2] as well as their important biological roles in nitrogen fixation and chemical defense.[3] Recent studies also proved that flavonoids possess a broad range of pharmacological properties, including antioxidant, anticancer, and anti-inflammatory activities[4,5]; hence received considerable therapeutic importance.

Except the differences on the structures of flavonoid aglycones and their glycosides, the great bioactive differences are also available. FAs are superior to their corresponding glycosides in various activities, due to the better solubility and oral bioavailability.[6,7] In addition, recent reports indicated that most FGs were hydrolyzed into their corresponding aglycones by human intestinal microflora after oral administration.[8,9] Teruaki et al.[10] found that baicalin could be absorbed as its hydrolyzed products - baicalein but not baicalin itself in the rat gastrointestinal tract. These previous studies show that baicalein possesses more distinctive pharmacological effects and metabolites safety than baicalin, so it is interesting to obtain FA for further chemical modification and preclinical studies. Unfortunately, natural FA is little in contrast to their respective glycosides. Total syntheses of FAs, such as baicalein and scutellarein, have been reported by Silvestri[11] and Chen[12] respectively, but they are unsuitable for industrial production due to their lengthy processes and low yields.

In the light of the natural abundance of FGs,[13] the conversion from FGs to their corresponding aglycones by bio- and chemical-transformations is required urgently. Although bioconversion seems more promising, their application has faced some difficulties, including substrate specificity, enzyme purification,

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maintenance of enzyme activity and high cost.[14–17] In comparison, the acid catalyzed cleavage of the glycosidic bond has been extensively used for glucosides hydrolysis in view of its universal versatility and maneuverability.[18] Different from glucosides, however, most studies have focused on the acid-catalyzed hydrolyses of glucuronides, because the special structure of glucuronide moiety is so stubborn that glucuronides are hard to be hydrolyzed under mild acid hydrolysis conditions. Much effort has been devoted to develop novel hydrolysis methods of glucuronide, but numerous glucuronides, such as scutellarin and baicalin are still hardly hydrolyzed unless under vigorous acid hydrolysis conditions. Although Li[19] and Tang[20] have independently examined the hydrolysis of scutellarin in aqueous mineral acid recently, both of their methods needed high temperature, long reaction time and without satisfied yields (mostly below 20%). In addition, the formation of byproducts associated with this protocol might occur, which involves an elaborate and tedious procedure; for example, the isomerization of flavonoids is formed due to a reversible Wessely–Moser rearrangement reaction (Fig. 1).[21] Consequently, alternative methods to hydrolyze FGs would be essential for the industrial preparation of FAs, including important chemical constituents in the pharmaceutical, cosmetic, and food industries. Until now, to the best of our knowledge, no appropriate approach on hydrolyzing flavonoid glucuronide into corresponding aglycone with simple purification and high yield processing has been reported.

We hypothesize that the proton of 5-carboxylic acid of flavonoid glucuronide might obstruct the first two steps of acid hydrolysis of glycoside based on theoretical calculations. Therefore, the protection for the carboxyl function in the chemical conversion of FGs to FAs was required. The present study aims to improve dilute-acid hydrolysis of the most FGs by protecting the carboxyl function and to develop a new universally sustainable method to ensure a convenient large-scale production of aglycones. The optimal C_5-substituent groups of glucuronides moiety (hereinafter denoted as FGM) were first chosen based on both the physical properties of glucuronides and the industrial feasibility by theoretical calculation using Gaussian program. The synthetic FGM was then subjected to dilute-acid hydrolysis for FA production. Additionally, the products of the hydrolysis process of FGM were examined and compared with that of glucuronide by the HPLC. The proportional amplification and substrate applicability of this method were also evaluated for the acid hydrolysis of scutellarin and other flavonoid glucuronides.

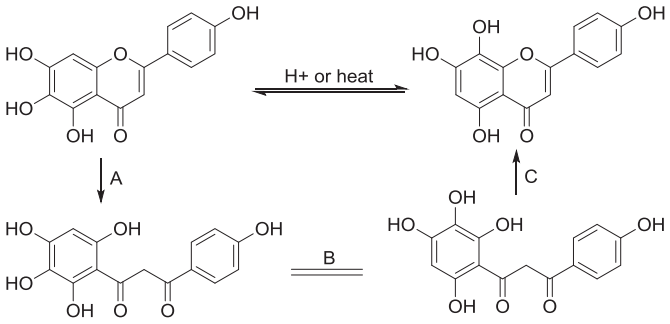


Fig. 1. Mechanism of Wessley-Mosher rearrangement reaction for scutellarein.

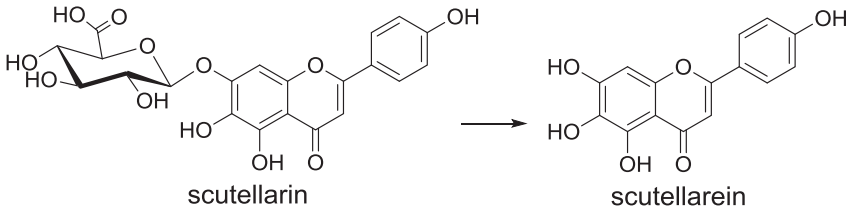
2. Results and discussion

2.1. Cause of glucuronide hydrolysis reaction problems and their solutions

2.1.1. The carboxyl of glucuronide is the "culprit"!

We chose scutellarin as the substrate and attempted to optimize the hydrolysis process in order to improve the demonstrated productivity of scutellarein. It should be noted that 90% EtOH was chosen for economical reason, and H_2SO_4 was used as the acid throughout our study because sulfuric acid can provide strong acidity and stable proton concentration in the acid hydrolysis reaction. Both of concentrations of sulfuric acid and reaction temperatures were evaluated. Indeed, only a 15% conversion was achieved using 0.5 mol/L H_2SO_4 at 120 °C for 48 h (Table 1, entry 5), which was consistent with the previous hydrolyses reaction performed under similar conditions.[20] Using a stronger acidic reaction media could not increase the conversion ratio of scutellarin, while scutellarein isomers might generate due to a reversible Wessely-Moser rearrangement reaction under such hard condition.[24] The rearrangement reaction could happen following several steps: (A) ring opening to the diketone, (B) bond rotation with the formation of a favorable acetylacetone-like phenyl-ketone interaction and (C) ring closure (Fig. 1).

Table 1
Optimization of hydrolysis conditions of scutellarin with sulfuric acid.[a]



Entry	H_2SO_4 (mol/L)	Solvent[b]	T (°C)	Time (h)	Conv. (%)[c]
1	1	90% Ethanol	90	24	4
2	2	90% Ethanol	90	24	5
3	3	90% Ethanol	90	24	8
4	3	90% Ethanol	100	48	10
5	3	90% Ethanol	120	48	15
6	4	90% Ethanol	120	48	16

[a] Unless otherwise stated, all reactions were carried out with scutellarin (0.5 mmol) and H_2SO_4 (15 mmol, **30 equiv**) under N_2 atmosphere.
[b] 90% Ethanol represent EtOH/H_2O = 90/10 (v/v).
[c] Conversion determined by HPLC analysis.