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Neuroprotective glucosides of magnolol and honokiol from microbial-specific glycosylation

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ABSTRACT

Thirteen new glucosides (**1**–**13**) of magnolol and honokiol were obtained from specific O-glycosylation by two filamentous fungi, *Cunninghamella echinulata* AS 3.3400 and *Rhizopus japonicus* ZW-4. The glucosides' structures were determined on the basis of extensive spectroscopic (HRESIMS, 1D and 2D NMR, and CD) analyses and a chemical method. *C. echinulata* appeared to transfer a glucosyl moiety to 2-OH of magnolol and honokiol, whereas *R. japonicus* preferred to regio-specifically transfer a glucosyl moiety to 4′-OH when honokiol was as the substrate. In addition, hydroxylation by *C. echinulata* and specific 6″-O-acylation of the introduced glucosyl moiety by *R. japonicus* were observed as minor reactions. Bioassay results indicated that glucosides **1**–**12** together with magnolol and honokiol at 10 μM attenuated the glutamate-induced toxicity in SK-N-SH cells to levels comparable to the results for MK-801, a positive control. However, the water-solubility of major glucosylated products (**1**, **8**, and **11**) increased greatly.

1. Introduction

Magnoliae cortex, the bark of the *Magnolia officinalis* Rhed. (Magnoliaceae) is a Chinese and Japanese crude drug used for the treatment of gastrointestinal disorders, anxiety and allergies.[1] Magnolol and honokiol (Fig. 1) are two bioactive biphenyl-type neolignans that are abundantly available in the medicinal plants *M. officinalis* and *M. obovata*.[2] Recently, they have been reported to exhibit multiple biological properties such as anti-cerebral ischemia, anti-neurodegenerative, anti-oxidative, anti-tumorigenic, anti-diabetic, anti-microbial, and anti-inflammation activities.[1,3–6] However, the low bioavailability of oral and intra-peritoneal administration limits their practical application.[7,8] It is important to develop novel pharmacological magnolol/honokiol derivatives with improved pharmacokinetics/pharmacodynamics properties and greater potential to treat numerous disorders. Therefore, their structural modification has been attracting much more attention and achieved numerous progresses by chemical approach.[9–12] However, some reactions including glycosylation, which is generally considered to enhance the water-solubility and bioavailability of a compound,[13] are not easily accessible by chemical methods. In such cases, biotransformation should be a versatile alternative.[14–18] There have been a number of reports on the glycosylation of natural products by enzymes and whole cell.[19,20] In this context, the microbial transformations of magnolol/honokiol have been systematically investigated in our group, and two filamentous fungi have been found to efficiently glycosylate magnolol and honokiol. In this report, we describe the glycosylation of magnolol and honokiol, the structural elucidation of the glycosides, and the neuroprotective activity against glutamate-induced toxicity in SK-N-SH cells.

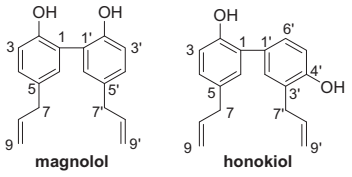

Fig. 1. The chemical structures of magnolol and honokiol.

2. Results and discussion

2.1. Microbial transformations of magnolol and honokiol

In this investigation, twenty-two strains of filamentous fungi, seven strains of actinomycetes, seven strains of bacteria and one

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yeast strain (*see* Table S1) were employed for the biotransformation of magnolol and honokiol. Analysis by TLC and HPLC-UV/MS showed that five filamentous fungal strains efficiently converted magnolol, and four strains efficiently converted honokiol, (*see* Fig. S1). By considering the yields, diversity and novelty of the products, *Cunninghamella echinulata* AS 3.3400 and *Rhizopus japonicus* ZW-4 were selected for the further scale-up biotransformation.

Following a standard two-stage fermentation protocol,[18] magnolol and honokiol were incubated with the respective cell cultures of the two strains for four days. The products were purified by the combination of silica-gel column chromatography and semi-preparative HPLC. In the case of magnolol transformation, five (**1**–**5**, Fig. 2) and three metabolites (**1**, **6**, **7**, Fig. 2) were isolated with *C. echinulata* and *R. japonicas*, respectively. For honokiol transformation, three (**8**–**10**, Fig. 3) and four (**8**, **11**–**13**, Fig. 3) metabolites were yielded with *C. echinulata* and *R. japonicas*, respectively. All of the metabolites were new glucosides, and their structures were identified on the basis of extensive spectroscopic data (HRESIMS, 1D and 2D NMR, and CD) and a chemical method. By comparing the structures and the yields of the products, it is concluded that *C. echinulata* can glycosylate magnolol and honokiol at 2-OH, whereas *R. japonicus* regio-specifically glycosylates magnolol at 2-OH and honokiol at 4′-OH. The major reaction was glycosylation, along with the minor reactions of hydroxylation by *C. echinulata* and specific 6″-O-acylation of the introduced glucosyl moiety by *R. japonicas*.

glycosidic linkage occurs at 2-OH, which was further supported by the HMBC correlation of the anomeric proton of the glucosyl moiety at δ_H 4.93 (H-1″) and C-2 (δ_C 152.7). Therefore, the structure of **1** was identified as magnolol-2-*O*-β-D-glucopyranoside.

The molecular weight of **2** was 16 amu higher than that of **1** by HR-ESI-MS analysis, suggesting the introduction of one additional hydroxyl in comparison with **1**. The ^{1}H and ^{13}C NMR data of **2** (Tables 1 and 2) were very similar to those of **1**, except for the disappearance of the 7-methylene signal (δ_H 3.32 and δ_C 38.7) in **1** and the appearance of an oxy-bearing methine (δ_H 4.98, δ_C 73.0) in **2**. The HMBC correlations of H-4, H-6, and H-8/C-7 indicated that the hydroxyl was introduced at C-7. The negative Cotton curve at 239 nm in its CD spectrum suggested an *R* configuration for C-7 according to benzene sector rule.[22] Accordingly, **2** was identified as (7*R*)-7-hydroxy-magnolol-2-*O*-β-D-glucopyranoside.

The molecular formula of **3** was established as $C_{24}H_{28}O_8$ by HR-ESI-MS analysis. The ^{1}H NMR data of **3** were similar to those of **1**, except that an allyl-group signal was replaced by a set of oxygenated propenyl-group signals [δ_H 6.50 (1H, d, J=16.0 Hz), 6.23 (1H, dt, J=4.8, 16.0 Hz), and 4.08 (2H, t, J=4.8 Hz)] (Table 1). The ^{13}C NMR spectrum of **3** also exhibited the oxygenated propenyl group signals [δ_C 128.1 (d), 128.9 (d), and 61.6 (t)] (Table 2). The key HMBC correlations (Fig. 4) of H-4 (δ_H 7.33) and H-6 (δ_H 7.23)/C-7 (δ_C 128.1) suggested that the reactions occurred on the glycosylated unit. Thus, **3** was identified as $\Delta^{7,8}$-7*E*-9-hydroxy-magnolol-2-*O*-β-D-glucopyranoside, a double-bond rearranged and hydroxylated derivative of **1**.

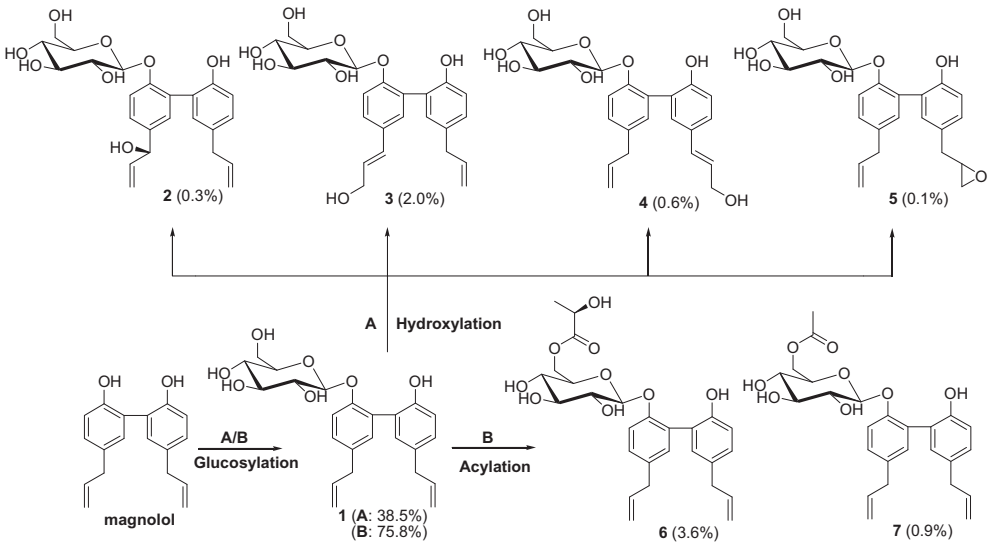

Fig. 2. The plausible biotransformation route for metabolites from magnolol (A: by *C. echinulata* AS 3.3400; B: by *R. japonicas* ZW-4; yields in parentheses).

The molecular formula of **1** was determined to be $C_{24}H_{28}O_7$ by positive HR-ESI-MS (*m*/*z* 451.1726 [M+Na]$^+$, calcd 451.1727 for $C_{24}H_{28}O_7Na$). The molecular weight of **1** was 162 amu higher than that of magnolol, suggesting the introduction of a hexosyl moiety via glycosylation. The ^{1}H and ^{13}C NMR data of **1** were similar to those of magnolol (Tables 1 and 2), except for the presence of an additional sugar moiety evidenced by the typical signals of an anomeric proton [δ_H 4.93 (1H, d, J=7.6 Hz)] and its corresponding anomeric carbon (δ_C 100.4). Acid hydrolysis of **1** yielded magnolol and one sugar. The sugar was identified as D-glucopyranose by GC–MS analysis, in which the retention time of the derivatives of the sugar residue and the standard sugar were compared, as described in the Experimental Section.[21] The β-anomeric configuration of the glucosyl moiety was assigned from the large coupling constant (J=7.6 Hz) of the anomeric proton. A downfield shift of C-2 (δ_C 152.7) in **1** compared with that in magnolol indicated that the

The HR-ESI-MS spectrum of **4** exhibited an ion peak at *m*/*z* 467.1673 [M+Na]$^+$, consistent with the molecular formula $C_{24}H_{28}O_8$. Compound **4** also had an oxygenated-propenyl-group signal instead of an allyl-group signal as was observed for **3**, the subtle differences in the chemical shifts may have resulted from the different location of the oxygenated propenyl group. The key HMBC correlations of H-4′ (δ_H 7.22) and H-6′ (7.25)/C-7′ (δ_C 128.6) suggested that the reactions occurred in the un-glucosylated unit. Hence, **4** was identified as $\Delta^{7',8'}$-7′*E*-9′-hydroxy -magnolol-2-*O*-β-D-glucopyranoside.

Compound **5** has the same molecular formula of $C_{24}H_{28}O_8$ as compounds **2**–**4** by combined analyses of HR-ESI-MS and NMR spectroscopic data (Tables 1 and 2). The ^{1}H NMR data were similar to those of **1**, except for the absence of the olefilic proton signals [δ_H 5.94 (1H, H-8′) and 5.04 (2H, H-9′)] of an allyl group and the presence of additional signals [δ_H 3.26 (1H, m), 2.52 (1H, m), and