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Original article

Microbial transformation of glycyrrhetinic acid and potent neural anti-inflammatory activity of the metabolites

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ABSTRACT

The microbial transformation of glycyrrhetinic acid (**1**) by *Cunninghamella blakesleana* CGMCC 3.970 led to the production of five new metabolites (**2–6**). The structures of the metabolites were determined by extensive spectroscopic (HR-ESIMS, 1D and 2D NMR) data analyses. The involved reactions exhibited specific hydroxylations at C-24, C-7, and C-15, and oxidation at C-3. Moreover, compounds **2**, **5**, and **6** showed significant neural anti-inflammatory activity by inhibiting lipopolysaccharide-induced NO production in mouse microglia BV2 cells with IC_{50} values of 0.76, 0.94, and 0.16 μmol/L, respectively.

1. Introduction

Glycyrrhetinic acid (GA, **1**, Fig. 1), a pentacyclic triterpenoid derivative of the glycyrrhizin, is one of the major constituents of the roots of *Glycyrrhiza uralensis* which is used in traditional Chinese medicine. Recently, it has been reported that GA exhibits diverse biological properties such as anti-inflammatory, antiulcerative, antiviral, antiallergic, immunomodulating, and antitumour activities [1,2]. Especially, carbenoxolone, a derivative of GA, has been successfully marked as a licensed medicine for the treatment of esophageal ulceration and inflammation [3]. Thus, the structural modification of GA for more potent agents has attracted much more attention, and numerous progresses has been achieved by microbial transformation [4–8]. Microbial biotransformation is regarded as a powerful approach for the structural modification of natural products with many advantages such as regio- and stereo-selectivity, mild reaction conditions, as well as avoiding complex protection and de-protection steps. Particularly, it can access the reaction that it is difficult to be carried out by chemical approaches [9–12]. Herein, we report the transformation of GA by *Cunninghamella blakesleana* CGMCC 3.970, the structural elucidation, and neural anti-inflammatory activity of the five new metabolites.

2. Results and discussion

2.1. Biotransformation

In this investigation, we aimed at obtaining new bioactive derivatives of GA through microbial transformation approach. TLC and HPLC-UV/MS analysis showed that ten filamentous fungal strains converted GA efficiently (Figs. S1 and S2 in Supporting information). By considering the yields, diversity and novelty of the products, *C. blakesleana* CGMCC 3.970 was selected for the further scale-up biotransformation investigation.

Following a standard two-stage fermentation protocol [13], GA (**1**) was incubated with the two-day-old cell cultures of *C. blakesleana* for seven additional days. Through various chromatography approaches, five new metabolites (**2–6**, Fig. 1) were isolated. Their structures were elucidated as 15α,24-dihydroxyglycyrrhetinic acid (**2**, 0.11%), 3-oxo-15α,24-dihydroxyglycyrrhetinic acid (**3**, 0.75%), 3-oxo-7β,24-dihydroxyglycyrrhetinic acid (**4**, 0.16%), 3-oxo-7β,15α,24-trihydroxyglycyrrhetinic acid

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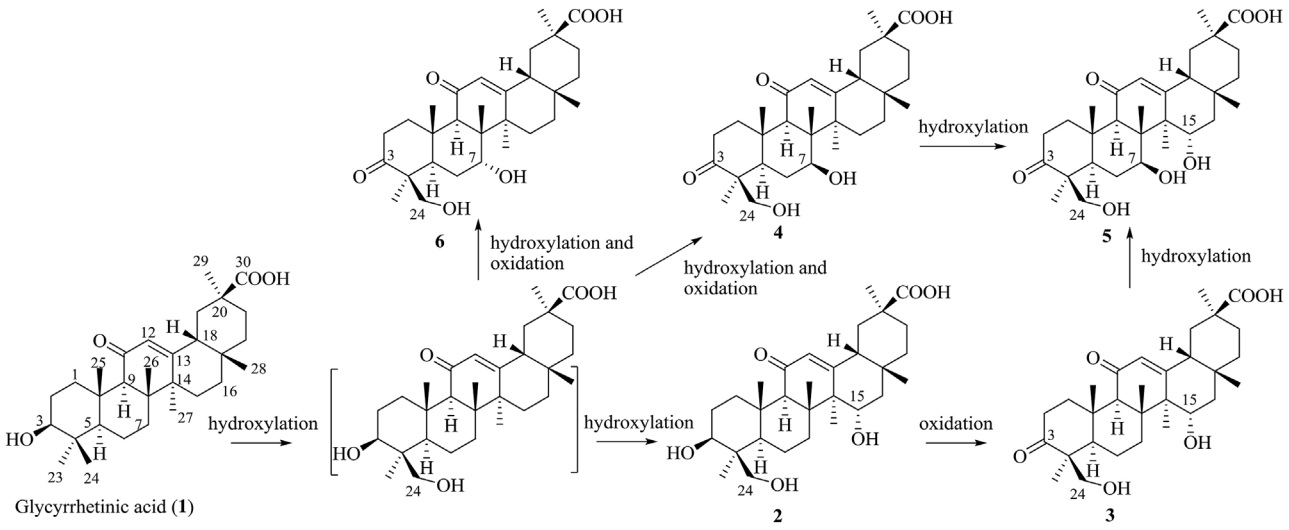

Fig. 1. Possible biotransformation pathway of glycyrrhetinic acid (**1**) by *C. blakesleana* CGMCC 3.970.

(**5**, 0.29%), and 3-oxo-7α,24-dihydroxyglycyrrhetinic acid (**6**, 1.27%) by interpreting various spectroscopic data. These results indicated that *C. blakesleana* CGMCC 3.970 could catalyze the specific hydroxylation at C-24, C-7, and C-15, and oxidation at C-3. Comparing the chemical structures of substrate (**1**) and the products (**2–6**), a plausible bioconversion route was proposed (Fig. 1).

2.2. Structural elucidation

The molecular formula of metabolite **2** was established as $C_{30}H_{46}O_6$ on the basis of HR-ESIMS (*m/z* 503.3380 $[M+H]^+$, calcd. for $C_{30}H_{47}O_6$, 503.3367). The UV spectrum showed absorption band at 250.5 nm. The IR spectrum showed the presence of hydroxyls (3343 cm^{-1}) and carbonyl (1644 cm^{-1}) groups. The extra 32 mass unit, compared to that of **1**, suggested the presence of two additional hydroxy groups. Analysis of the ^1H and ^{13}C NMR spectroscopic data of **2** revealed structural similarity to those of **1** (Tables 1 and 2), except that the appearance of one additional oxygenated methine proton at δ_H 4.11 (dd, J = 5.4, 11.4 Hz, H-15) and a hydroxymethyl protons at δ_H 4.00 (d, J = 10.8 Hz, H-24a) and 3.30 (d, J = 10.8 Hz, H-24b). These assignments of the oxygenated methine and hydroxymethyl protons as H-15 and H_2-24, respectively, were confirmed by the HMBC correlations of H-15 (δ_H 4.11)/C-14, C-16, and C-27; and H_2-24 (δ_H 3.30 and 4.00)/C-3, C-4, and C-23. The relative configuration of H-15 in **2** was determined by its 1D-NOESY spectrum, in which the NOESY correlations of H-15 with H_3-26 (δ_H 1.06) and H_3-28 (δ_H 0.74) indicated their *syn*-orientation. Besides, in the CD spectrum, the characteristic Cotton effect (CE) of α,β-unsaturated ketone was observed, the configuration at C-9

Table 1
The ^1H NMR data of compounds **2–6** (600 MHz, J in Hz, CD_3OD).

Position	2	3	4	5	6
1	2.64 (br d, 13.8, Hβ); 0.91 (m, Hα)	2.91 (ddd, 3.0, 6.0, 10.8, Hβ); 1.30 (m, Hα)[a]	2.75 (ddd, 4.8, 7.8, 10.8, Hβ); 1.40 (m, Hα)	2.93 (ddd, 3.0, 6.0, 10.8); 1.32 (m)[a]	2.93 (ddd, 3.0, 6.0, 10.8, Hβ); 1.35 (m, Hα)[a]
2	1.71 (m); 1.56 (m)	2.69 (ddd, 6.0, 10.8, 13.2); 2.15 (ddd, 3.0, 4.8, 13.2)	2.48 (ddd, 7.8, 10.8, 10.8); 2.31 (ddd, 3.6, 7.8, 10.8)	2.72 (ddd, 6.0, 10.8, 13.2); 2.20 (m)[a]	2.73 (ddd, 6.0, 10.8, 13.8, Hα); 2.18 (m, Hβ)[a]
3	3.25 (m)[a]				
5	0.78 (m)[a]	1.28 (m)[a]	2.01 (dd, 1.8, 10.2)	1.40 (m)[a]	1.35 (m)[a]
6	1.82 (m); 1.58 (m)	1.60 (m)[a]	1.57 (m) 1.51 (ddd, 2.4, 4.8, 13.2)	1.78 (ddd, 3.0, 4.8, 13.2, Hα); 1.70 (dd, 12.6, 13.2, Hβ)	1.65 (m)[a]; 1.35 (m)[a]
7	1.82 (m); 1.63 (br d, 13.8)	1.80 (m)[a]; 1.58 (m)[a]	4.09 (dd, 4.8, 10.8)	3.98 (dd, 4.8, 10.8)	3.95, m
9	2.34 (s)	2.44 (s)	2.54 (s)	2.40 (s)	2.44 (s)
12	5.59 (s)	5.64 (s)	5.65 (s)	5.67 (s)	5.60 (s)
15	4.11 (dd, 5.4, 11.4)	4.12 (dd, 4.8, 11.4)	1.90 (m); 1.38 (m)	4.27 (dd, 5.4, 11.4)	1.88 (m); 1.35 (m)[a]
16	1.99 (dd, 11.4, 12.0);1.82 (m)	2.00 (dd, 11.4, 12.0); 1.14 (m)[a]	2.10 (m); 1.24 (m)	2.06 (dd, 11.4, 12.0, Hα); 1.24 (m, Hβ)[a]	1.68 (m)[b]; 1.35 (m)[a]
18	2.15 (br d, 11.4)	2.18 (m)[a]	2.22 (m)	2.20 (m)[a]	2.18 (m)[a]
19	1.82 (m); 1.30 (m)	1.84 (m); 1.28 (m)	1.60 (dd, 11.4, 11.4); 1.45 (m)	1.83 (br d, 12.6); 1.63 (dd, 12.6, 12.6)	1.80 (br d, 12.6); 1.68 (m)[a]
21	1.82 (m); 1.38 (m)	1.84 (m); 1.58 (m)	2.10 (m); 0.96 (m)	1.91 (br d, 8.4); 1.32 (m)[a]	2.08 (m)[b]; 0.95 (m)[a]
22	1.68 (m)[a]	1.84 (m)	1.90 (m)	1.32 (m)[a]	2.08 (m)[b]; 1.53 (m)[a]
23	1.01 (s)	1.06 (s)	0.87 (s)	1.13 (s)	1.12 (s)
24	4.00 (d, 10.8); 3.30 (d, 10.8)	3.92 (d, 11.4); 3.43 (d, 11.4)	3.58 (d, 10.8); 3.25 (d, 10.8)	3.93 (d, 11.4); 3.48 (d, 11.4)	3.95 (d, 10.8); 3.46 (d, 10.8)
25	1.03 (s)	0.96 (s)	1.07 (br s)	1.11 (s)	1.29 (s)
26	1.06 (s)	1.25 (s)	0.78 (s)	0.73 (s)	1.09 (s)
27	1.30 (s)	1.30 (s)	1.45 (s)	1.36 (s)	1.41 (s)
28	0.74 (s)	0.79 (s)	1.11 (s)	1.29 (s)	0.78 (s)
29	1.11 (s)	1.12 (s)	1.20 (s)	1.12 (s)	1.09 (s)

[a] Overlapped with other signals.

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