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Hydroxylation of nigranoic acid to 6β-hydroxynigranoic acid by *Caryospora carllicarpa* YMF1.01026

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Abstract

Microbial transformation of nigranoic acid by *Caryospora carllicarpa* YMF1.01026 afforded the new derivative 6β-hydroxynigranoic acid. Its structure was elucidated by spectroscopic methods.

Keywords: Nigranoic acid; 6β-Hydroxynigranoic acid; Microbial transformation

Nigranoic acid is an A ring-secocycloartene triterpinoid produced by plants belonging to the genera Schisandra [1,2] and has been reported to possess a variety of biological activities, including cytotoxic activity toward Leukemia and Hela cells, and inhibition of expression HIV reverse transcriptase and polymerase. Recently, we isolated much nigranoic acid from *Schisandra propinqua* prompted us to obtain more analogues of further nigranoic acid for structure–activity relationship studies. Microbial transformation is an important tool for structural modification of organic compounds, especially natural with complicated structures [3,4]. It can be used to synthesize the analogues of the natural compounds, which are difficult to obtain by other means [5]. In the present paper, we report the isolation and identification of one new metabolite **2** from nigranoic acid **1** (see Fig. 1) by microbial transformation of the substrate with a *Caryospora carllicarpa* YMF1.01026 culture.

C. carllicarpa YMF1.01026 was inoculated in 250 mL flask contained 70 mL PDB (potato dextrose broth) medium. Four hundred milligrams of nigranoic acid was dissolved in methanol and equally accede to 10 flasks, when the strain was inoculated in, then the disposal was cultured at 28 °C for 12 days. The cultures of *C. carllicarpa* YMF1.01026 containing nigranoic acid were combined (700 mL) and filtered. The filtrate was exhaustively extracted four times with ethyl acetate and 298 mg transformational residue of 6β-hydroxynigranoic acid was obtained from the filtrate. The EtOAc extract was purified employing a combination of silica gel CC and Sephedex LH-20 chromatography to afford **2** as white amorphous powder (1.5 mg) (Fig. 1).

Compound **2** was isolated as a white amorphous powder. Its HRESIMS had the $[M + Na]^+$ ion at *m/z* 509.3249 (calcd. 509.3242), suggesting the molecular formula of $C_{30}H_{46}O_5Na$. The ^{13}C NMR spectrum showed an additional

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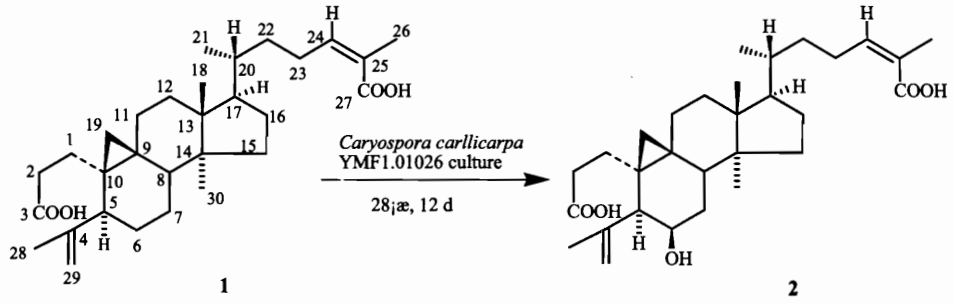

Fig. 1. The microbial transformation pathway of nigranoic acid by *Caryospora carllicarpa* YMF1.01026 culture.

oxygenated methine signal at δ_C 69.6. Compared to the corresponding data for nigranoic acid, the C-7 (δ_C 34.5), C-5 (δ_C 48.9) and C-28 (δ_C 114.3) signals shifted downfield by 9.8, 1.3 and 1.6 ppm, respectively, whereas C-4 (δ_C 147.8) and C-8 (δ_C 41.1) signals shifted upfield by 3.4 and 8.6 ppm, respectively, suggesting the hydroxylation on C-6. This conclusion was confirmed by the COSY correlation of H-5 (δ_H 2.42, d, 4.21Hz) with H-6 (δ_H 4.13, m) and the HMBC correlations of H-6 with C-5 (δ_C 48.9), C-10 (δ_C 28.3) and C-7 (δ_C 34.5). This metabolite **2** was the result of the regio- and stereoselective oxidation of the methelene group at C-6 of substrate **1** from the α face giving a (6R)-hydroxyl derivative, which, due to the poor selectivity, is difficult to achieve by chemical means. The R configuration at C-6

Table 1
The NMR data of compounds **1** and **2** in CD_3OD[a] (δ ppm, J Hz)

No.	**1**		**2**	
	δ_C	δ_H	δ_H	δ_C
1	20.8 (m); 1.43 (m)	30.8 t	2.09 (m); 1.40 (m)	31.1 t
2	2.53 (m); 2.28 (m)	33.0 t	2.51 (m); 2.30 (m)	33.1 t
3		178.2 s		178.4 s
4		151.2 s		147.8 s
5	2.53 (m)	47.6 d	2.42 (d, 4.21)	48.9 d
6	1.57 (m); 1.18 (m)	29.6 t	4.13 (m)	69.6 d
7	1.41 (m); 1.20 (m)	26.7 t	2.55 (m); 1.66 (m)	34.5 t
8	1.62 (m)	49.7 d	1.72 (m)	41.1 d
9		23.1 s		23.8 s
10		28.9 s		28.3 s
11	1.57 (m); 1.36 (m)	37.5 t	1.60 (m); 1.30 (m)	37.4 t
12	1.57 (m); 1.20 (m)	37.3 t	1.60 (m); 1.21 (m)	37.4 t
13		46.8 s		47.1 s
14		50.6 s		49.9 s
15	1.75 (m)	34.8 t	1.73 (m)	34.9 t
16	1.98 (m); 1.38 (m)	29.5 t	1.98 (m); 1.40 (m)	29.6 t
17	1.68 (m)	53.9 d	1.62 (m)	54.2 d
18	1.02 (s)	20.4 q	1.04 (s)	19.8q
19	0.78 (d, 3.4, βH); 0.46 (d, 3.4, αH)	31.6 t	1.15 (m); 0.58 (d, 4.0, αH)	31.8 t
20	1.46 (m)	37.8 d	1.45 (m)	37.7 d
21	0.96 (d, 6.1)	19.3 q	0.96 (s)	19.1 q
22	2.18 (m); 1.35 (m)	28.6 t	2.18 (m); 1.33 (m)	28.3 t
23	2.53 (m); 2.43 (m)	28.1 t	2.51 (m); 2.41 (m)	28.0 t
24	5.89 (t, 6.7)	144.8 d	5.95 (t, 6.7)	144.6 d
25		128.9 s		129.4 s
26	1.90 (s)	20.7 q	1.90 (s)	20.7 q
27		172.1 s		172.8 s
28	4.88 (s); 4.77 (s)	112.7 t	5.06 (m); 5.02 (m)	114.3 t
29	1.73 (s)	21.6 q	1.87 (s)	21.4 q
30	1.04 (s)	19.3 q	1.09 (s)	19.1 q

[a] The NMR data for compounds **1** and **2** were recorded on Bruker AM-500 MHz.

could easily be deduced from the coupling constant value 4.21 Hz of H-5, which were reconfirmed by the presence of NOESY correlation between H-5 and H-6. Thus, the structure of **2** was determined to be 6β-hydroxyl nigranoic acid.

Compound **2**: white amorphous powder; $[\alpha]_D^{28.0}$ + 25.7 (*c* 0.65, MeOH); IR (film) *ν* 3425, 2927, 2857, 1705, 1641, 1460, 1380, 1273, 1073, 900 cm^{-1}; TOF MS *m/z* (rel. int): 509 $[M + Na]^+$; HRTOF-MS *m/z*: 509.3249 $[M + Na]^+$ (calcd. for $C_{30}H_{46}O_5Na$ 509.3242). The NMR spectral data see Table 1.

Microorganisms are one of the most efficient biocatalytic agents known with the capacity to metabolize a wide range of substrates. It is possible to increase the yield by monitoring and changing multivariate components of the biotransformation process, along with improved biological testing procedures, spectroscopic techniques, and nano-scale synthetic processes. Microbial transformations would be an increasingly viable tool in medicinal chemistry.

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