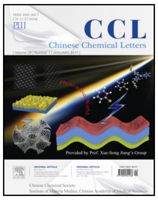
Chinese Chemical Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet



Original article

Triterpenoids and phenolics from the fruiting bodies of *Inonotus hispidus* and their activations of melanogenesis and tyrosinase

Qing Ren[a,b], Xue-Ying Lu[a], Jian-Xin Han[a,b], Haji Akber Aisa[a], Tao Yuan[a,*]

[a] *The Key Laboratory of Plant Resources and Chemistry of Arid Zone, Chinese Academy of Sciences, State Key Laboratory of Xinjiang Indigenous Medicinal Plants Resource Utilization, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi 830011, China*
[b] *University of Chinese Academy of Sciences, Beijing 100049, China*

ARTICLE INFO

Article history:
Received 8 October 2016
Received in revised form 15 November 2016
Accepted 30 November 2016
Available online xxx

Keywords:
Inonotus hispidus
Lanostane triterpenoids
Phenolic compound
Melanogenesis
Tyrosinase

ABSTRACT

Two new 24-methyl lanostane triterpenoids, hispindic acids A and B (**1** and **2**), and a new phenolic compound, hispinine (**7**), along with nine known compounds (**3–6**, and **8–12**), were isolated from the fruiting bodies of *Inonotus hispidus*. Their structures were elucidated based on the extensive analysis of spectroscopic data (NMR and HRMS). Hispindic acid A (**1**) possesses an unusual formyl group at C-30. Compounds **1**, **3–4**, and **8** showed stronger activate abilities of melanogenesis and tyrosinase in B16 melanoma cells than those of positive control, 8-methoxypsoralen, at 50 μmol/L.

1. Introduction

Inonotus hispidus (Bull. Ex Fr.) Karst is a parasitic fungus in the family Hymenochaetaceae. It is preferably living on a variety of deciduous trees such as malus, fraxinus, sorbus and quercus. *I. hispidus* mainly distributed in the Northeast regions and Xinjiang province of China, and was used as a traditional medicine for the treatment of dyspepsia, cancer, diabetes and stomach problems in these regions [1]. Previous chemical investigations of this species have reported the presence of a considerable quantity of yellow-brown pigments, *e.g.* hispidin, hispolon, and some hispidin derivatives dimmers, which exhibited antimicrobial, antioxidant, and anti-inflammatory activities [2–7]. In our interest in discovering bioactive compounds from Xinjiang (China) indigenous medicinal fungus, three new compounds including two 24-methyl lanostane triterpenoids (**1** and **2**) and a phenolic compound (**7**), together with nine known compounds (**3–6**, and **8–12**) (Fig. 1), were isolated and identified from the methanolic extract of the fruiting bodies of *I. hispidus*. Moreover, all of the isolates were evaluated for their activations of melanogenesis and tyrosinase, the related targets of vitiligo. Herein, the isolation and structural elucidation, as well as the evaluation of activating melanogenesis and tyrosinase, were present.

2. Results and discussion

The total of 12 compounds (**1**–**12**) including three new ones, were isolated from the fruiting bodies of *I. hispidus*. Herein, the structural elucidation of the new compounds is presented.

Compound **1** was obtained as white amorphous powder, its molecular formula was determined as $C_{31}H_{48}O_4$ by HRESIMS at m/z 483.3489 $[M-H]^-$ (calcd. for $C_{31}H_{47}O_4$, 483.3474). The IR absorptions showed the presence of hydroxyl (3445 cm^{-1}) and carbonyl (1706 cm^{-1}) functionalities. The ^1H NMR spectrum of **1** (Table 1) showed six methyl groups signals at δ_H 0.71, 0.73, 0.87, 0.98 (each 3H, s), 0.95 (d, 3H, $J = 7.5$ Hz), 0.96 (d, 3H, $J = 6.6$ Hz), a terminal double bond signals at δ_H 4.72, 4.63 (s, each 1H), a formyl group signal at δ_H 9.36 (s, 1H) and an oxygen-bearing methine signal at δ_H 3.00 (m, 1H). The ^{13}C NMR (Table 1) and HSQC spectra revealed the presence of 31 carbon resonances comprising six methyls, 11 methylenes, five methines and nine quaternary carbons (two of which are carbonyl and three olefinic carbons). The aforementioned data implied compound **1** to be a 24-methyl lanostane triterpenoid. Comparison of the ^1H NMR and ^{13}C NMR data of **1** with those of compound **3** (eburicoic acid) [8], indicated that they were structurally similar, the only difference being the group at C-30. The methyl group of position-30 was replaced by a formyl group in **1**, which was supported by the NMR data of CHO-30 (δ_H 9.36, δ_C 199.8), and verified by the HMBC correlations (Fig. 2a) from H-30 to C-14 (δ_C 67.2) and C-15 (δ_C 22.3). The overall structure of compound **1** was further confirmed by ^1H–^1H COSY

---

* Corresponding author.
E-mail address: yuantao@ms.xjb.ac.cn (T. Yuan).

http://dx.doi.org/10.1016/j.cclet.2016.12.010

Please cite this article in press as: Q. Ren, et al., Triterpenoids and phenolics from the fruiting bodies of *Inonotus hispidus* and their activations of melanogenesis and tyrosinase, Chin. Chem. Lett. (2016), http://dx.doi.org/10.1016/j.cclet.2016.12.010

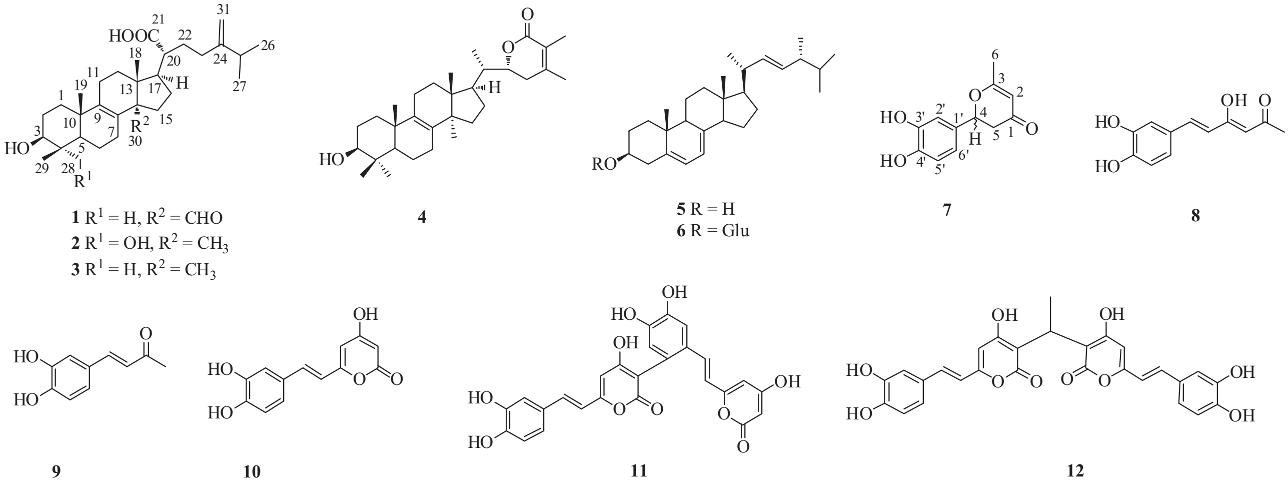


Fig. 1. Structures of compounds **1**–**12**.

and HMBC data. The relative configurations of compound **1** were established by examination of the NOESY experiment (Fig. 2b) and coupling constant. The β-orientation of 3-OH was determined on the basis of coupling constant of H-3 (J = 10.3, 5.0 Hz) [9]. NOESY correlations from: H-3 to H-1α, H-5 and H_3-28; H-30 to H-12α; H-12α/H-17, indicated that they were co-facial, and in an α-orientation. Consequently, the NOESY correlations from: H_3-19 to H_3-29, H-12β and H_3-18; H_3-18 to H-20, showed they were β-oriented. The above NOESY data indicated that compound **1** possessed the same relative configurations with eburicoic acid. Therefore, the structure of compound **1** was elucidated as 24-exomethylene-3β-hydroxy-30-oxo-lanost-8-en-21-oic acid, assigned the trivial name hispindic acid A.

Compound **2** was obtained as a white, amorphous powder with a molecular formula of $C_{31}H_{50}O_4$, as determined by HR-ESIMS at m/z 485.3661 $[M-H]^-$ (calcd. for $C_{31}H_{49}O_4$, 485.3631). Analysis of ^1H NMR and ^{13}C NMR data of compound **2** suggested that the structure of **2** was also similar to that of compound **3** (eburicoic

Table 1
^1H NMR and ^{13}C NMR Spectroscopic data of compounds **1**, **2** and **7**.[a]

Position	**1**[b]		**2**[b]		**7**[c]	
	δ_H(mult,J, Hz)	δ_C	δ_H(mult,J, Hz)	δ_C	δ_H(mult,J, Hz)	δ_C
1	1.72 (m), 1.22 (m)	36.5	1.61 (m), 1.09 (m)	35.4		194.4
2	1.52 (m, 2H)	28.7	1.51 (m, 2H)	27.6	5.40 (s)	103.7
3	3.00 (dd, 10.3, 5.0)	77.7	3.44 (m)	70.7		176.3
4		39.8		42.4	5.29 (dd, 13.9, 3.6)	80.9
5	0.93 (m)	50.9	1.38 (m)	42.3	2.82 (dd, 16.6, 13.9) 2.47 (dd, 16.6, 3.6)	41.3
6	1.59 (m), 1.45 (m)	19.0	1.55 (m), 1.40 (m)	17.8	2.05 (3H, s)	19.7
7 (1′)	1.87 (m), 1.46 (m)	27.3	1.92 (m), 1.31 (m)	26.9		129.6
8 (2′)		122.7		133.8	6.86 (brs)	113.3
9 (3′)		145.1		134.8		145.1
10 (4′)		38.7		36.7		145.6
11 (5′)	2.27 (m), 2.13 (m)	22.4	2.01 (m), 1.91 (m)	20.8	6.77 (d, 8.5)	114.8
12 (6′)	1.90 (m), 1.51 (m)	29.0	1.55 (m), 1.37 (m)	28.9	6.74 (brd, 8.5)	117.9
13		46.2		44.3		
14		67.2		49.5		
15	1.98 (m), 1.35 (m)	22.3	1.55 (m), 1.15 (m)	30.5		
16	1.75 (m), 1.35 (m)	27.2	2.03 (m), 1.93 (m)	26.0		
17	1.38 (m)	49.8	1.93 (m)	47.0		
18	0.73 (s, 3H)	17.7	0.69 (3H, s)	16.1		
19	0.98 (s, 3H)	20.4	0.93 (3H, s)	19.8		
20	2.13 (m)	48.0	2.11 (m)	48.0		
21		177.8		177.4		
22	1.53 (m), 1.42 (m)	31.3	1.60 (m), 1.49 (m)	31.1		
23	1.88 (m, 2H)	32.5	1.91 (2H, m)	32.0		
24		155.8		155.2		
25	2.15 (m)	34.4	2.19 (m)	33.7		
26	0.95 (d, 3H, 7.5)	22.8	0.96 (d, 3H, 6.5)	22.1		
27	0.96 (d, 3H, 6.6)	22.7	0.97 (3H, d, 7.0)	22.0		
28	0.87 (s, 3H,)	29.2	3.33 (overlapped) 3.10 (dd, 10.3, 5.1)	64.9		
29	0.71 (3H, s)	17.0	0.56 (3H, s)	12.9		
30	9.36 (s)	199.8	0.84 (3H, s)	24.5		
31	4.72 (s), 4.63 (s)	108.0	4.72 (s), 4.64 (s)	107.2		

[a] Recorded at 600 or 150 MHz for ^1H and ^{13}C, resp.
[b] In DMSO-d_6.
[c] In CD_3OD.

Please cite this article in press as: Q. Ren, et al., Triterpenoids and phenolics from the fruiting bodies of *Inonotus hispidus* and their activations of melanogenesis and tyrosinase, Chin. Chem. Lett. (2016), http://dx.doi.org/10.1016/j.cclet.2016.12.010