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ARTICLE

Regio- and stereo- selective oxidation of β-boswellic acids transformed by filamentous fungi

Cite this: DOI: 10.1039/xoxxoooooox

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Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Biotransformation of 11-keto-β-boswellic acid (KBA) and acetyl-11-keto-β-boswellic acid (AKBA) catalyzed by two fungal strains (*Cunninghamella elegans* AS 3.1207 and *Penicillium janthinellum* AS 3.510) was performed in present investigation. Eleven transformed products (**1-11**) were isolated, and accurately identified by various spectral methods. Among them, eight products (**1-4** and **8-11**) are novel. Two microorganisms used in our experiments demonstrated the favourable capability of stereo- and regio-hydroxylation at the non-active position for boswellic acid skeletons (KBA and AKBA). *P. janthinellum* AS 3.510 preferred to catalyze hydroxylation reaction at C-21α position, especially for AKBA with the yield of 35.7%. While, *C. elegans* AS 3.1207 preferred to catalyze hydroxylation reaction of C-21β, especially for KBA with the yield of 55.2%. The major metabolite **1** exhibited the potent anti-inflammatory activity in the *in vitro* bioassay.

Introduction

Structural modification of complex natural products is usually necessary to enhance bioactivity, reduce toxicity or improve their physical and chemical properties. The classical chemistry approaches as the routine solution, often encounter some regio- or stereo-selectivity problems. Moreover, multi-step reactions including protection or deprotection, usually result in low yield of the target products. Biotransformation is usually regarded as an alternative tool to modify a substrate into a specific product with its great capabilities to perform chemical transformations with high regio- and stereoselectivity that cannot be accessed by standard transformations.[1] Nowaday, it has been widely used in pharmaceutical synthesis and is attracting more and more interests of researchers.[2] The filamentous fungi, bacteria and cultured plant suspension cells were commonly used as the biocatalyst systems. Many studies demonstrated that a large number of filamentous fungi could catalyze the complex and diverse reactions for natural products, especially such as triterpenoids and steroids, which was usually difficult to complete by the traditional chemical synthesis. For example, *Fusariumsolani* had the excellent capability to catalyze isomerization and dehydrogenation of bufadienolides at 3-OH,[3] and *Alternaria alternate* had great specificity for the 12-Hydroxylation of bufadienolides.[4] And several filamentous fungi could catalyse cycloastrgenol to produce novel derivatives.[5] To our knowledge, hydroxylation as a common biocatalysis reaction, was observed in biotransformation of triterpenoids.[6,7] And it was reported that hydroxylation usually happened at C-1, C-7, C-12 and C-15 sites of A-D rings in the skeletons of complex triterpenoids.[8-11] However, in previous report, hydroxylation on C-20, C-21 and C-22 positions of E-ring was really rare and typically low-yielding.[12-14] So there is a great challenge to find the appropriate microorganism for realization of a desired biotransformation reaction at some non-activated positions of the triterpene skeleton such as E-ring, with relatively high yields.

11-keto-β-boswellic acid (KBA) and acetyl-11-keto-β-boswellic acid (AKBA) as the natural triterpenes, were abundantly available from *Boswellia serrata*, a kind of deciduous tree centered in the dry regions of tropical Africa and India.[15,16] KBA and AKBA possessed the unique pentacyclic ring skeleton, and exhibited the significant bioactivities such as anti-inflammatory,[17] anti-arthritic diseases,[18] treatment of asthma[19] and anticancer[20]. Compared to NSAIDS, it is associated with better tolerability[21] and devoid of typical

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†Electronic Supplementary Information (ESI) available: The spectra including 1D-, 2D-NMR, HRESIMS of compounds **1-11**. See DOI: 10.1039/b000000x/

adverse effects[22]. They could suppress leukotriene formation via selective inhibition of 5-lipoxygenase,[23] increasing the activity of NF-κB[24] and inhibiting COX-1 product synthesis[25]. However, poor absorption and extensive metabolism may play a crucial role inlimiting the bioavailability of 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid.[26] Their structural modification is thus of great necessity for further evaluation of structural activity relationship. But some chemical positions that could be modified by the chemical methods were extremely limited, due to an unactivated molecule of triterpene. Recently, our research had exhibited that some novel biotransformed products of AKBA and KBA with hydroxylation at non functionalized groups of substrates by *C. blakesleana* AS 3.970, also suggested biotransformation was a vital approach in structural modification of AKBA and its derivatives.[27,28] Therefore, microbial transformation of xenobiotics is a very useful approach to expand the chemical diversity of these natural products. Moreover, microbial transformation is suggested to be a rational way to convert KBA and AKBA to those desired products.

In present work, the high selective biotransformation of AKBA and KBA by two strains of filamentous fungi, namely *Cunninghamella elegans* AS 3.1207 and *Penicillium janthinellum* AS 3.510, was investigated. Eleven metabolites were isolated and purified from the fungal broth, and their structures were fully characterized by NMR and HRESIMS. The region- and stereo-specific hydroxylation reactions of two fungal strains for AKBA and KBA were discussed. In addition, their inhibitory activities on lipopolysaccharide (LPS)-induced nitric oxide (NO) production were also investigated.

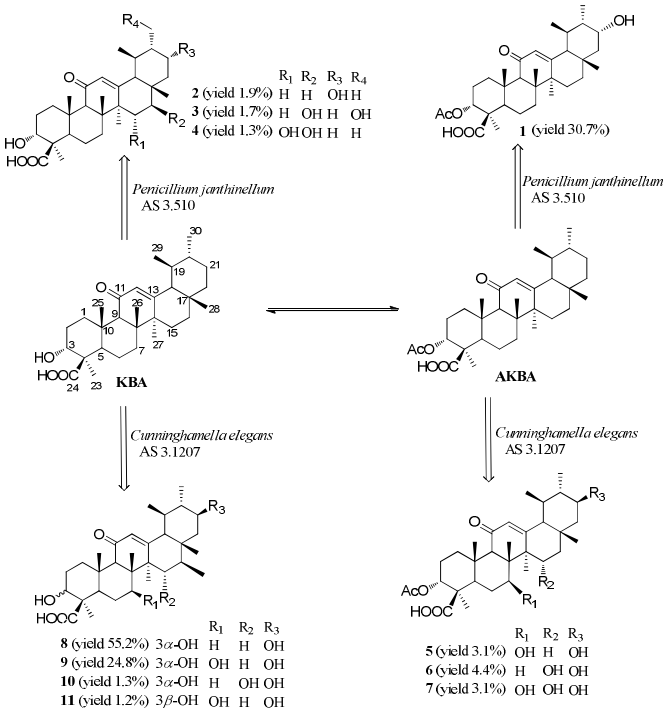
Results and discussion

Preliminary screening test exhibited that KBA and AKBA could be efficiently metabolized by two fungal strains: *Cunninghamella elegans* AS 3.1207 and *Penicillium janthinellum* AS 3.510. And then preparative experiments of these two stains were carried out for obtaining the metabolites, respectively. After 5 days of incubation, the culture supernatant was extracted with ethyl acetate, and the crude materials were subjected to ODS column chromatography and semi-preparative HPLC to yield compound **1** (AKBA by *P. janthinellum*), compounds **2-4** (KBA by *P. janthinellum*), metabolites **5-7** (AKBA by *C. elegans*), and metabolites **8-11** (KBA by *C. elegans*), respectively (Scheme 1). Their structures were identified by various spectroscopic methods including 2D-NMR analyses. The ^{1}H NMR and ^{13}C NMR data of metabolites **1-11** were also listed in Tables 1, 2, and 3.

Biotransformation of boswellic acids by *Penicillium janthinellum* AS 3.510

AKBA – selective biotransformation by *P janthinellum* with the high yield.The molecular formula of **1** was deduced to be $C_{32}H_{48}O_5$ using HRESIMS *m/z* 609.3014 $[M+2H+Br]^+$. Its NMR spectrum indicated that it is a monohydroxylated derivative of AKBA. An additional oxygenated carbon resonance at δ_C 76.2 was observed in the ^{13}C-NMR spectrum, which was correlated to a proton signal at δ_H 4.56 in the HMQC experiment. In the HMBC spectrum, the carbon signal of δ_C 76.2 had the long-range correlation with Me-30 (δ_H 1.29), which suggested that C-21 was oxygenated. The NOE enhancement of H-20 (δ_H 1.22) with Me-29 (δ_H 0.80) and H-21 (δ_H 4.56) implied that 21-OH should be α-oriented. Therefore, compound **1** was identified as 21α-hydroxy-3-acetyl-11-keto-β-boswellic acid.

KBA - diversity biotransformation by *P. janthinellum*. Compound **2** was obtained as white powder with molecular formula of $C_{30}H_{46}O_5$ based on HRESIMS *m/z* 567.2916 $[M+2H+Br]^+$. Its ^{1}H and ^{13}C-NMR spectra were similar to those of KBA, except for an additional oxygen-bearing methine signal at δ_C 76.2 and a new methine proton at δ_H 4.56, all of which suggested the introduction of a hydroxyl group in the molecule. In the HMBC spectrum, the long-range correlation of carbon signal of δ_C 76.1 with Me-30 (δ_H 1.29) indicated that the hydroxyl group was located at C-21. In the NOESY spectrum, the NOE effects of H-21 (δ_H 4.56) with H-20 (δ_H 1.20) and Me-28 (δ_H 0.77) implied α orientation of 21-OH. Therefore, compound **2** was identified as 21α-hydroxy-11-keto-β-boswellic acid.



Scheme 1. Biotransformation of *Penicillium janthinellum* AS 3.510 and *Cunninghamellaelegans* AS 3.1207 on acetyl-11-keto-β-boswellic acid (AKBA) and 11-keto-β-boswellic acid (KBA).

Compound **3** was obtained as a white powder. Its molecular formula of $C_{30}H_{46}O_6$ was confirmed by HRESIMS *m/z* 503.3148 $[M+H]^+$.Compared with KBA, two oxygenated carbon signals at δ_C 77.8, 64.6 were observed in the ^{13}C-NMR spectrum, while the characteristic signal of Me-30 was disappeared. In the HMBC spectrum, the proton signal of δ_H 3.70 had the HMBC correlations with Me-28 (δ_C 25.6) and C-22 (δ_C 21.0), indicating that the hydroxyl group was located at C-16. Meantime, the long-range correlation of δ_H 3.90/C-21 (δ_C 34.8) suggested the hydroxyl group was substituted at C-30. The NOE effect of H-16 (δ_H 3.70) with Me-27 (δ_H 1.31) proved that 16-OH was β-oriented. Therefore, compound **3** was identified as 16β,30-dihydroxy-11-keto-β-boswellic acid.

Metabolite **4** was obtained as white powder. Its HRESIMS showed a *quasi*-molecular ion peak $[M+H]^+$ at *m/z* 503.3313, suggesting a molecular formula of $C_{30}H_{46}O_6$. The ^{1}H NMR spectrum exhibited two additional oxygen-bearing methane protons at δ_H 3.65 and δ_H 3.44. And its ^{13}C NMR spectrum showed two additional oxygenated carbon signals at δ_C 74.7 andδ_C82.8. In the HMBC spectrum, the long-range correlations

from δ_H 3.44 to C-22 (δ_C 22.5), Me-28 (δ_C 25.8), C-17 (δ_C 40.0) and C-15 (δ_C74.7) were observed, while bearing oxygen carbon

Table 1. ^{1}H-NMR spectral data of compounds **1-6** (Pry-d_5, 600 MHz, δ in ppm, J in Hz).

H	1	2	3	4	5	6
1a	1.55 m	2.08 m	2.09 m	2.12 m	1.52 m	3.02 m
1b	2.95 m	3.05 m	3.08 m	3.08 m	2.96 m	1.62 m
2a	1.84 m	2.01 m	1.99 m	2.02 m	1.83 m	1.87 m
2b	2.72 m	2.92 m	2.91 m	2.92 m	2.75 m	2.76 m
3	5.93brs	4.73 brs	4.73 brs	4.74 brs	5.98 brs	5.97 s
5	1.74 m	2.08 m	2.10 m	2.12 m	1.88 m	1.84 m
6a	2.07 m	2.12 m	2.12 m	2.12 m	2.78 m	2.16 m
6b	2.36 m	2.41 m	2.48 m	2.43 m	2.53 m	2.33 m
7a	1.39 m	1.39 m	1.45 m	1.45 m	4.45d(7.8)	2.27 m
b	1.73 m	1.72 m	1.77 m	1.77 m	---	2.33 m
9	2.68 s	2.75 s	2.81 s	2.81 s	2.74 s	2.86 s
12	5.77 s	5.75 s	5.83 s	5.82 s	5.90 s	5.92 s
15a	1.03 m	0.98 m	1.17 m	3.65 t	2.10 m	4.54 dd (10.8, 6.0)
15b	1.71 m	1.68 m	1.81 m		2.45 m	
16a	1.08 m	1.08 m	3.7 dd (12.0,2.0)	3.44 d (6.0)	1.14 m	1.69 m
16b	1.88 m	1.87 m			2.09 m	2.36 m
18	1.56 m	1.53 m	1.67d(10.8)	1.69 m	1.66 m	1.69m
19	1.57 m	1.56 m	2.08 m	1.69 m	1.70 m	1.76 m
20	1.22 m	1.20 m	1.31 m	1.26 m	1.10 m	1.14 m
21a	4.56 m	4.56 m	2.23 m	1.44 m	3.68 m	3.69 m
21b			2.33 m	1.79 m		
22a	1.57 m	1.57 m	1.74 m	2.03 m	1.54 m	1.54 m
22b	2.57 m	2.57 m	2.06 m	2.12 m	2.04 m	2.12 m
23	1.52 s	1.81s	1.80 s	1.81s	1.49 s	1.51 s
25	1.61 s	1.72s	1.75 s	1.75 s	1.64 s	1.69 s
26	1.20 s	1.22 s	1.33 s	1.30	1.53 s	1.42 s
27	1.24 s	1.16 s	1.31 s	1.27 s	1.52 s	1.61 s
28	0.75 s	0.77 s	1.21 s	1.84 s	0.93 s	0.94 s
29	0.80 d(6.0)	0.76d(6.0)	0.91d (6.0)	0.82d (4.8)	0.9d (6.0)	0.96 s
30	1.29 s	1.29d (5.4)	3.90 m	1.29 s	1.3 d (6.0)	1.28d (6.0)
Ac	2.10s				2.06 s	2.09 s

of δ_C 74.7 showed cross peaks with H-16 (δ_H 3.44) and Me-28 (δ_C 25.8). These evidences confirmed that two hydroxyl groups were substituted at C-15 and C-16, respectively. The β orientation of 16-OH was established according to the NOE enhancement between H-16 (δ_H 3.44) and Me-27 (δ_H 1.27). While NOE correlation of H-15 (δ_H 3.65) with Me-26 (δ_H 1.30)was also observed, indicating an α-orientation of 15-OH. On the basis of above analyses, the structure of **4** was determined as 15α,16β-dihydroxy-11-keto-β-boswellic acid.

Biotransformation of boswellic acids by *Cunninghamella elegans* AS 3.1207

AKBA – diversity biotransformation by *C. elegans*. Compound **5** was obtained as a white powder. Its HR-ESIMS provided a molecular formula of $C_{32}H_{48}O_7$, according to a *quasi*-molecular ion peak $[M-H]^-$ at *m/z* 543.3321. Its ^{1}H-NMR spectrum showed two additional oxygen-bearing methine protons at δ_H 4.45 and δ_H 3.68. The ^{13}C NMR spectrum showed two additional carbon signals at δ_C 72.9 and δ_C 70.3. In the HMBC spectrum, the long-range correlations of δ_C 70.3/Me-30 (δ_H 1.30), H-22a (δ_H 1.54) and H-22b (δ_H 2.04), confirmed that the hydroxyl group should located at C-21 position. In the ^{1}H-^{1}H COSY spectrum, the proton of δ_H 4.45 had the correlations with H-6a (δ_H 2.78), H-6b (δ_H 2.53) and H-9 (δ_H2.74) suggesting compound **5** possessed 7-hydroxyl group. In the NOESY spectrum, the NOE enhancements of H-7 (δ_H 4.45) with Me-27 (δ_H 1.52) indicated that 7-OH should be in β-orientation. Meantime, the NOE enhancement of H-21 (δ_H 3.68) with H-19 (δ_H 1.70) exhibited the β-orientation of 21-OH. Therefore, compound **5** was identified as 7β,21β-dihydroxy-3-acetyl-11-keto-β-boswellic acid.

Analyses of the spectra of compound **6** and **7** indicated that they both were oxygenated derivatives of AKBA, with two and three hydroxyl moieties respectively. The positions of hydroxyl groups and stereochemistry could be elucidated by the 2D-NMR data. In the HMBC spectrum of **6**, the long-range correlations between δ_H 4.54 and Me-27 (δ_C 15.3), C-16 (δ_C 40.5), C-8 (δ_C 46.9) and C-14 (δ_C 50.0), suggested that hydroxylation occurred at C-15 position. In addition, the carbon signal of δ_C 70.2 showed the HMBC correlations with H-22a (δ_H 1.54), H-22b (δ_H 2.12), Me-29 (δ_H 0.96) and Me-30 (δ_H 1.28), which indicated that a hydroxyl group was located at C-21. Meantime, the NOE effects of H-15 (δ_H 4.54) with Me-26 (δ_H 1.42) and Me-28 (δ_H 0.94), indicated a hydroxyl group of C-15 should be in α-orientation. Similarly, 21-OH was established as β-oriented by the NOE enhancement between H-21 (δ_H 3.69) and Me-30 (δ_H 1.28) in the NOESY experiment. Therefore, the structure of compound **6** was determined as 15α,21β-dihydroxy-3-acetyl-11-keto-β-boswellic acid. The planar structure of **7** was determined as 7,15,21-trihydroxy-3-acetyl-11-keto-β-boswellic acid on the basis of the long-range correlations of δ_H 4.50 (H-7)/Me-26 (δ_C 13.0) ,C-14 (δ_C 51.3), δ_H 4.65 (H-15)/Me-27 (δ_C 15.3), C-16 (δ_C 38.4), C-8 (δ_C 51.8), δ_C70.1 (C-21)/H-22a (δ_H1.57), H-22b (δ_H 2.14), Me-28 (δ_H 0.95) and Me-30 (δ_H 1.29). The relative configurations of hydroxyl groups were established as 7β,15α,21β by the NOE correlations of H-7 (δ_H 4.50)/Me-27 (δ_H 1.60), H-5 (δ_H 1.92) and H-9 (δ_H 2.81),H-15 (δ_H 4.65)/Me-26 (δ_H 1.55) and Me-28 (δ_H 0.95),H-21 (δ_H 3.71)/Me-30 (δ_H 1.29). Thus, compound **7** was determined as 7β,15α,21β-trihydroxy-3-acetyl-11-keto-β-boswellic acid.

KBA – biotransformation by *C. elegans* with high yield. Compound **8** exhibited a *quasi*-molecular ion peak at $[M+H]^+$ *m/z* 487.3328 in the HRESIMS, corresponding to its molecular of $C_{30}H_{46}O_5$. Its ^{13}C NMR spectrum exhibited one additional oxygen-bearing carbon signal at δ_C 70.2. In the HMBC spectrum, C-21 (δ_C70.2) had the long-range correlations with Me-30 (δ_H 1.29), H-22a (δ_H 1.56) and H-22b (δ_H 2.06), which suggested that it possessed 21-hydroxyl group. The β-orientation of 21-OH was established according to the NOE enhancement between H-21 (δ_H 3.67) and Me-30 (δ_H 1.29). Thus, the structure of **8** was determined as 21β-hydroxy-11-keto-β-boswellic acid.

Compound **9** was isolated as a colorless crystal in MeOH. The molecular formula $C_{30}H_{46}O_6$ of **9** was determined by using HR-ESI-MS ($[M-H]^-$ *m/z* 501.3217). Its ^{1}H-NMR spectrum showed two additional oxygen-bearing protons at δ_H 4.48 and δ_H 3.68. The ^{13}C NMR spectrum showed two additional carbon signals at δ_C 73.1 and δ_C 70.3. In the HMBC spectrum, the long-range correlations of H-7

(δ_H 4.48) with Me-26 (δ_C 12.5) and C-14 (δ_C 45.9) were observed. At the same time, the carbon signal of δ_C 70.3 had the HMBC

Table 2. ^{1}H-NMR spectral data of compounds **7-11** (Pry-d_5, 600 MHz, δ in ppm, J in Hz).

H	7	8	9	10	11
1a	1.57 m	2.07 m	2.06 m	2.14 m	1.21 m
1b	3.01 m	3.07 m	3.06 m	3.10 m	3.19 m
2a	1.86 m	1.98 m	2.02 m	2.02 m	1.98 m
2b	2.73 m	2.92 m	2.91 m	2.94 m	2.01 m
3	5.95 brs	4.73 brs	4.73 brs	4.73 brs	3.44 dd(4.8, 4.2)
5	1.92 d(12.0)	2.09 m	2.26 d (12.0)	2.18 m	1.32 m
6a	2.59 m	2.12 m	2.81 m	2.16 m	2.57 m
6b	2.76 m	2.41 m	2.56 m	2.48 m	2.66 m
7a	4.50 dd(10.8, 4.2)	1.44 m	4.48 dd(11.4, 4.2)	2.34 m	4.42 dd(5.4, 3.6)
b		1.76 m	2.83 s	2.35 m	
9	2.81 s	2.79 s	5.90 s	2.92 s	2.65 s
12	5.98 s	5.80 s	2.09 m	5.91 s	5.91 s
15a	4.65 dd (11.4, 5.4)	1.06 m	2.46 m	4.54 d (6.6)	2.08 m
15b		1.76 m	1.13 m		2.45 m
16a	1.76 m	1.07 m	2.07 m	1.69 m	1.17 m
16b	2.37 m	1.98 m	1.70 m	2.34 m	2.03 m
18	1.72 m	1.59 m	1.70 m	1.65 m	1.69 m
19	1.73 m	1.58 m	1.12 m	1.73 m	1.74 m
20	1.17 m	1.08 m	3.68 m	1.12 m	1.13 m
21a	3.71 m	3.67 m		3.68 m	3.70 m
21b			2.06 m		
22a	1.57 m	1.56 m	1.56 m	1.53 m	1.59 m
22b	2.14 m	2.06 m	1.79 s	2.11m	2.08 m
23	1.52 s	1.81 s	1.82 s	1.80 s	1.73 s
25	1.68 s	1.74 s	1.59 s	1.80 s	1.71 s
26	1.55 s	1.28 s	1.45 s	1.46 s	1.53 s
27	1.60 s	1.24 s	0.92 s	1.55 s	1.58 s
28	0.95 s	0.85 s	0.87 d (6.6)	0.94 s	0.92 s
29	0.94 s	0.83 d (4.8)	1.29 d (6.6)	0.90 d (6.6)	0.9 d (6.6)
30	1.29 s	1.29 s		1.26 d (6.6)	1.32 d (6.6)
Ac	2.10 s				

correlations with H-20 (δ_H 1.12), H-22a (δ_H 2.06), Me-28 (δ_H 0.92) and Me-30 (δ_H 1.29), respectively. These evidences suggested that two hydroxyl groups should be located at C-7 and C-21, respectively. In the NOESY spectrum, H-7(δ_H 4.48) had the NOE enhancement with Me-27 (δ_H 1.45), and H-21 (δ_H 3.68) had the NOE effect with Me-30 (δ_H 1.29), all of which indicated 7-OH and 21-OH are all in β-orientations. Therefore, the structure of metabolite **9** was confirmed as 7β,21β-dihydroxy-11-keto-β-boswellic acid.

Compound **10** was obtained as white powder. Its molecular formula of $C_{30}H_{46}O_6$ was established from HRESIMS ($[M+H]^+$ *m/z* 503.3148). Comparing with the ^{13}C-NMR spectrum of KBA, two additional carbon signals at δ_C 70.2 and δ_C 66.9 were observed, which suggested that compound **10** should be a dihydroxylated derivative of KBA. The HMBC correlations of proton signal at δ_H 4.54 with C-16 (δ_C 40.5) and Me-27 (δ_C 15.1) suggested that hydroxylation occurred at C-15. In addition, the carbon signal of δ_C 70.2 showed the long-range correlations with Me-30 (δ_H 1.26) and H-22 (δ_H 1.53) which confirmed the hydroxyl group was located at C-21. In the NOESY spectrum, the NOE enhancement of H-15 (δ_H 4.54) with Me-26 (δ_H 1.46) and Me-28 (δ_H 0.49) indicated 15-OH should be α-orientated. Similarly, the NOE enhancement of H-21 (δ_H 3.68) with Me-30 (δ_H 1.26), indicated that 21-OH should be in β-orientation. Therefore, compound **10** was defined as 15α,21β-dihydroxy-11-keto-β-boswellic acid.

Compound **11** was obtained as a white powder. The molecular formula of $C_{30}H_{46}O_6$ was established from HRESIMS ($[M+H]^+$ *m/z* 503.3313). Its ^{1}H-NMR spectrum showed three additional oxygen-bearing methine protons at δ_H 4.42, δ_H 3.70 and δ_H 3.44, and the characteristic methine proton of H-3 was absent. The ^{13}C-NMR spectrum showed three additional carbon signals atδ_C72.9, δ_C70.3 and δ_C 78.1. In HMBC spectrum, the long-range correlations of δ_C 72.9 with Me-26 (δ_H 1.53), H-9 (δ_H 2.65), H-6a (δ_H 2.57) and H-5 (δ_H 1.32) were observed. At the same time, the carbon signal of δ_C 70.3 had the HMBC correlations with Me-30 (δ_H 1.32), H-22a (δ_H 1.59), H-22b (δ_H 2.08) and Me-28 (δ_H 0.92). Meantime, the proton signal of δ_H 3.44 correlated with Me-23 (δ_C 24.7) and C-4 (δ_C 49.1).These evidences suggested that three hydroxyl groups should be located at C-7, C-21 and C-3. The 3-OH was deduced to be in β-configuration by NOE enhancement of H-3 (δ_H 3.44) with H-5 (δ_H 1.32) and Me-23 (δ_H 1.73). In addition, H-7 (δ_H 4.42) had the NOE enhancement with Me-27 (δ_H 1.58), Me-30 (δ_H 1.32), and H-21 had the NOE effect with Me-30 (δ_H 1.29) and H-19 (δ_H 1.74), all of which indicated 7-OH and 21-OH are β-orientated. On the basis of above analyses, compound **11** was identified as 3-*epi*-7β,21β-dihydroxy-11-keto-β-boswellic acid.

Biotransformation features of the two fungal strains

The biotransformation features of the two fungal strains (*P. janthinellum* AS 3.510 and *C. elegans* AS 3.1207) showed the significant region and stereo-selective hydroxylation at the non-active sites of AKBA and KBA.

Incubation of AKBA with *P. janthinellum* AS 3.510 displayed the potent region and stereo-selectivity hydroxylation to produce a novel and rare hydroxylated product (**1**) with 21α-OH as the sole product, which was difficult to obtained by chemical transformation of triterpenoids or steroids at C-21, due to chemical steric hindrance of Me-30. And its yield was as high as 30.7%. However, after incubation with *P. janthinellum* AS 3.510, KBA (the deacetylation product of AKBA),was converted to its 21α-hydroxyl (**2**, 1.9% yield), 15α,16β-dihydroxyl (**4**, 1.3% yield) and 16β,30-dihydroxyl derivatives (**3**, 1.7% yield), respectively. It is particularly noteworthy that *P. janthinellum* AS 3.510 had the significant specificity between AKBA and KBA. And when 3-OAc group was existed in the chemical structures, *P. janthinellum* AS 3.510 exhibited significant capability of α-hydroxylation at C-21 with the excellent selectivity. However, when the acetyl group was disappeared from the chemical structures, *P. janthinellum* AS 3.510 exhibited significant capability of α-hydroxylation at C-21 with the excellent selectivity. However, when the acetyl group was disappeared from the chemical structure, only trace amounts of hydroxylated derivative at C-21α was obtained. These evidences indicated that 3-OAc group would be directly

related to the hydroxylation capabilities of *P. janthinellum*, which could be applied to the hydroxylation of C-21. *C. elegans* AS 3.1207 was found to transform AKBA to produce a series of hydroxylation derivatives. The main reaction sites were C-7, C-15 and C-21 positions to obtain products 5 (3.1% yield), **6** (4.4% yield) and 7 (3.1% yield), respectively. While, biotransformation of KBA by *C. elegans* AS 3.1207 could yield four metabolites (**8**-**11**). The main hydroxylation selectively occurred at C-21 position, and then dihydroxylation at the various sites such as C-7 and C-15 were observed with increasing biotransformation time. The derivatives **8** and **9**, were produced as major products with 55.2% and 24.8% yields, respectively. The yields of other trace derivatives **10** and **11** were only 1.3% and 1.2%, respectively.

Our results indicated that biotransformation was an effective approach to produce some novel compounds which are difficult to be synthesized by classical chemical means. In addition, each of the two fungal strains showed biocatalytic preference, which could be used to synthesize various oxygenated derivatives of KBA and AKBA. The introduction of hydroxyl groups in the molecule structures of metabolites catalyzed by *C. elegans* AS 3.1207, were mainly at 21*β*. However, major metabolite transformed by *P. janthinellum* AS 3.510 had the additional hydroxyl groups which always substituted at 21*α* position in the high yield. In addition, *C. elegans* AS 3.1207

Table 3.^{13}C-NMR spectral data of compounds (Pry-d_5, 150 MHz, δ in ppm).

C	1	2	3	4	5	6	7	8	9	10	11
1	35.4t	35.1t	35.1t	35.1t	35.2t	35.5t	35.0t	35.1t	34.9t	35.5t	40.0t
2	24.4t	27.6t	27.6t	27.6t	24.6t	24.4t	24.5t	27.5t	27.7t	27.7t	29.5t
3	74.1d	70.4d	70.4d	70.4d	74.0d	74.1d	73.8d	70.4d	70.2d	70.4d	78.1d
4	47.0s	48.3s	48.3s	48.3s	48.1s	47.0s	46.7s	48.3s	48.0s	48.3s	49.1s
5	50.8d	49.2d	49.3d	49.3d	48.1d	50.8d	47.6d	49.3d	46.3d	49.2d	53.6d
6	19.6t	19.8t	19.9t	19.9t	31.3t	20.0t	29.6t	19.9t	31.5t	20.2t	31.5t
7	33.1t	33.4t	33.4t	33.4t	72.9d	36.7t	71.7d	33.5t	73.1d	37.1t	72.9d
8	45.3s	45.4s	45.6s	45.6s	51.0s	46.9s	51.8s	45.5s	51.0s	47.0s	50.6s
9	60.8d	61.2d	61.2d	61.3d	61.2d	61.0d	61.4d	61.2d	61.6d	61.4d	61.3d
10	38.0s	38.4s	38.4s	37.8s	38.2s	38.2s	38.2s	38.4s	38.6s	38.4s	38.1s
11	198.9s	199.2s	199.4s	199.4s	198.7s	199.1s	198.2s	199.2s	200.0s	199.5s	198.6s
12	131.1d	131.2d	131.0d	131.1d	131.3d	131.5d	131.6d	131.2d	131.3d	131.6d	131.3d
13	164.2s	164.2s	164.2s	164.0s	165.0s	165.4s	165.3s	164.5s	164.8s	165.1s	165.0s
14	43.9s	44.0s	44.8s	44.8s	45.9s	50.0s	51.3s	44.0s	45.9s	50.0s	45.9s
15	27.4t	27.4t	27.2t	74.7d	31.6t	66.9d	66.5d	27.6t	31.6t	66.9d	31.5t
16	28.5t	28.5t	77.8d	82.8d	29.5t	40.5t	38.4t	28.9t	29.5t	40.5t	29.2t
17	35.3s	35.4s	39.8s	40.0s	35.5s	35.5s	35.0s	35.3s	35.6s	35.5s	35.6s
18	58.3d	58.3d	59.1d	58.4d	59.9d	59.3d	59.8d	58.7d	59.9d	59.3d	59.9d
19	38.4d	38.4d	33.7d	37.8d	38.8d	38.5d	38.6d	38.4d	38.8d	38.6d	38.8d
20	46.0d	46.1d	45.8d	44.7d	47.8d	47.6d	47.7d	47.7d	47.8d	47.6d	47.8d
21	76.2d	76.2d	34.8t	27.2t	70.3d	70.2d	70.1d	70.2d	70.3d	70.2d	70.3d
22	48.3t	48.3t	21.0t	22.5t	50.9t	50.7t	50.6t	51.0t	51.0t	50.7t	51.0t
23	24.5q	25.6q	25.6q	25.6q	21.2q	24.4q	24.3q	25.6q	25.4q	25.5q	24.7q
24	179.0s	180.5s	180.0s	180.5s	179.0s	179.0s	178.5s	180.4s	180.4s	180.5s	180.5s
25	13.9q	14.2q	14.3q	14.3q	12.5q	14.1q	13.8q	14.2q	14.3q	14.4q	14.6q
26	18.5q	18.6q	18.6q	18.6q	12.5q	19.2q	13.0q	18.6q	12.5q	19.3q	12.4q
27	20.5q	20.5q	20.6q	20.5q	20.6q	15.3q	15.3q	20.5q	20.5q	15.1q	20.6q
28	28.5q	28.5q	25.6q	25.8q	28.9q	29.5q	29.5q	28.7q	28.9q	29.6q	28.9q
29	17.5q	17.5q	17.1q	17.6q	17.7q	17.7q	16.2q	17.5q	17.6q	17.6q	17.6q
30	16.4q	16.4q	64.6t	16.4q	16.1q	16.2q	17.7q	16.2q	16.1q	16.2q	16.2q
$CH_3\underline{CO}$	170.4q				21.2q	21.2q	21.2q				
$\underline{CH_3}CO$	21.2s				170.4s	170.4s	170.3s				

and *P. janthinellum* AS 3.510 could be used as bioreactors to obtain C-21 hydroxylation metabolites of KBA and AKBA rapidly.

The biotransformation time-course of KBA by *C. elegans* AS 3.1207 and AKBA by *P. janthinellum* AS 3.510 was investigated in the present work. Three major metabolites with relatively high yields were listed in Table 4. Compound **1** reached the highest yield of 30.7% within 48 h by *P. janthinellum* AS 3.510. And the monohydroxylation product (**8**) was the initial metabolite of KBA by *C. elegans* AS 3.1207, reached the highest yield of 55.2% in 48 h. And then, the final conversion rate of product **9** determined to be the highest of 24.8% after incubation of 96 h. Our results indicated the *C. elegans* AS 3.1207 and *P. janthinellum* AS 3.510 could be used as bioreactors to produce the hydroxylated derivatives of C-21α or β with relatively high yields.

Table 4. Biotransformation yields of products (**1**, **8** and **9**) by *P. janthinellum* AS 3.510 and *C. elegans* AS 3.1207.

Time (h)	1	8	9
24	21.8%	30.9%	10.1%
48	30.7%	55.2%	14.0%
72	21.9%	37.2%	21.7%
96	17.2%	35.7%	24.8%

Inhibitory Effects on Nitric Oxide (NO) Production in LPS-Activated Macrophages

The inhibitory effects on nitric oxide production in LPS-induced macrophages of KBA, AKBA and metabolites **1-11** were evaluated. As shown in Table 5, compound **1** showed potent inhibitory ability on NO production with the IC_{50} value 7.7 μM, as well as had no influence on the cell viability by MTT method.

Table 5. Inhibitory effects of compounds **1-11** against LPS-induced NO production in RAW264.7 macrophages.

Compound	IC_{50} value (μM)	Cell viability[a]
KBA	8.6	9.38±0.33
AKBA	19.2	89.12±1.65
1	7.7	103.23±2.37
2	23.6	79.12±3.43
3	>100	109.64±0.36
4	72.6	95.18±1.38
5	84.1	108.25±1.78
6	>100	107.12±4.17
7	>100	98.21±3.43
8	69.3	102.83±0.62
9	>100	104.72±4.05
10	>100	97.07±0.82
11	41.6	104.38±2.15
MINO[b]	37.32	12.0±2.63

[a] The cell viability of RAW264.7 cells in the presence of derivatives at a dose of 100 μM after a period of 24h.

[b] Minocycline (MINO) was used as the positive control for NO production.

Experimental

General experimental produces

^{1}H-, ^{13}C- and 2D- NMR were performed in pridine-d_5 on a Bruker DRX-600 spectrometer (600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR). The chemical shifts and coupling constants (J) were given in δ (ppm) and hertz (Hz), respectively. IR spectra were obtained with an Avatar 360 FT-TR spectrophotometer. HR-ESIMS spectra were measured on Q-TOF MS (Micromass). Preparative liquid chromatography was performed on a UItimate 3000 HPLC instrument equipped with photodiode array detector (PAD). Samples were separated on a DIONEX Acclaim 120 column (Ø250 mm×4.6 mm). All solvents including ethyl acetate, petroleum ether (60-90ºC) and acetone are A.R. grade and were obtained from Tianjin Kemiou Chemical Reagent Company (Tianjing, China). Methanol and acetonitrile for HPLC analysis are chromatographic grade (Merck, Darmstadt, Germany). Silica gel (200-300 mesh) for column chromatography was purchased from Qingdao Marine Chemical Group, Qingdao, China.

Substrate

AKBA and KBA used for biotransformation experiments were isolated from *Boswelliacarteri* by the author (C. Wang). After meshed, the raw material (1 kg) was extracted with ethanol (95%) by ultrasonic for 5 times. The extracted solution was concentrated under reduced pressure and a portion of residue (40 g) was subjected to a silica gel column (1000 g) eluted with petroleum ether/EtOAc (20:1-0:1) to afford five fractions (I-V). Following TLC detection, Fr. IV was then separated by silica column chromatography eluted with petroleum ether/EtOAc (8:1-0:1) to give crude AKBA and KBA, which were then purified by HPLC instrument. The purities of these two substrates were determined to be 98% by HPLC analysis.

Microorganisms and culture media

A total of **17** different fungal strains, including *Absidia coerulea* AS 3.3389, *Absidia coerulea* AS 3.3538, *Actinomucor elegans* AS 3.2778, *Aspergillus niger* AS 3.739, *Aspergillus niger* AS 3.795, *Aspergillus niger* AS 3.1858, *Aspergillus niger* AS 3.4627, *Chaetomium globosum* AS 3.4254, *Cunninghamella elegans* AS 3.1207, *Cunninghamella elegans* AS 3.2028, *Cunninghamella echinulata* AS 3.3400, *Fusarium avenaceum AS* 3.4594, *Mucor rouxianus* AS 3.3447, *Penicillium melinii* AS 3.4474, *Penicillium janthinellum* AS 3.510, *Rhizopus oryzae* AS 3.2380 and *Syncephalastrum racemosum* AS 3.264, were used in this experiment. All of microorganisms were purchased from Chinese General Microbiological Culture Collection Center in Beijing, China. All culture and biotransformation experiments using filamentous fungi were performed in potato medium which was prepared as follows: 200 g of peeled potato was boiled for 30 min in water and the solution was filtered. The filtrate was added glucose (20 g) and then diluted water to 1L. The media was sterilized at 121 ºC and 1.06 kg/cm^2 for 30 min.

Preliminary biotransformation for screening

The fungal strains from the agar slant were directly transferred to 250 ml Erlenmeyer flasks containing 100 ml of potato medium and then incubated at 30 ºC and 170 rpm for 24 h. For each flask, 0.2 ml of acetone containing substrate AKBA or KBA (1.5mg) was administered to the culture and the incubation was carried out for another 5 days. Two control groups were performed under the same culture conditions without organism or substrates.

Preparative scale biotransformation experiments

The fungal strains (*P. janthinellum* AS 3.510 and *C. elegans* AS 3.1207) were sub-cultured for four times on potato culture medium to obtain maximal biotransformation activities before used for preparative scale experiments. Preparative experiments were carried out in 1000 ml Erlenmeyer flasks containing 400 ml of potato medium. Substrate in acetone (50 mg/ml) was added to the cultures, which was pre-cultured for 36 h. Other procedure and culture conditions were identical with these of screening experiments.

Extraction and purification

The cultures were pooled and filtered, then the filtrate was acidified by 1.0% HCl solution and extracted with an equal volume of ethyl acetate for four times. The organic layer was concentrated *in vacuo*. The isolation process mainly contained two steps, preliminary purified on ODS column and further purified by HPLC.

Incubated with AKBA by *P. janthinellum* AS 3.510: A total of 90 mg of AKBA was added to the cultures. After concentrated, the EtOAc extract (0.7 g) was purified by ODS column and eluted with MeOH-H_2O-TFA (65:35:0.03, *v/v*) in gradient manner to give 10 fractions. The fraction (NO.4) was further purified by semi-preparative HPLC to give **1** (31.5 mg, 35.7%).

Incubated with KBA by *P. janthinellum* AS 3.510: A total of 150 mg of KBA was added to the cultures. After concentrated, the EtOAc extract (0.9 g) was subjected to ODS column eluted with MeOH-H_2O (20:80-100:0) in a gradient manner to afford 25 fractions. Fr. 4 was subjected to preparative HPLC and eluted with MeOH-H_2O-TFA (57:43:0.03, *v/v*) to give compound **2** (2.9 mg, 1.9%). Fr. 6 was purified by semi-preparative HPLC to give compound **3** (2.6 mg, 1.7%) eluted with MeOH-H_2O-TFA (46:54:0.03, *v/v*), Fr. 7 was subjected to preparative HPLC and eluted with MeOH-H_2O-TFA (47:53:0.03, *v/v*) to give compound **4** (2.0 mg, 1.3%).

Incubated with AKBA by *C. elegans* AS 3.1207: A total of 160 mg of AKBA was added to the cultures. After concentrated, the EtOAc extract (0.9 g) was subjected to ODS column eluted with MeOH-H_2O (20:80-100:0) in a gradient manner to afford 25 fractions. Fr. 9 was subjected to preparative HPLC and eluted with MeCN–H_2O–TFA (35: 65:0.03, *v/v*) to give compound **7** (5 mg, 3.1%). Fr. 13 was purified by semi-preparative HPLC to give compound **6** (7 mg, 4.4%) eluted with MeCN–H_2O–TFA (37:63:0.03, *v/v*). Fr. 17 was purified by semi-preparative HPLC to give compound **5** (5 mg, 3.1%) eluted with MeOH-H_2O-TFA (46:54:0.03, *v/v*).

Incubated with KBA by *C. elegans* AS 3.1207: A total of 170 mg of KBA was added to the cultures. After 5 days of incubation, 1.0 g of ethyl acetate extract was obtained from the culture supernatant and was subjected to ODS column eluted with MeOH-H_2O (20:80-100:0) in a gradient manner to give30 fractions. Fr. 12 was subjected to preparative HPLC and eluted with MeCN–H_2O–TFA (35:65:0.03, *v/v*) to give compound **10** (2.2 mg, 1.3%) and **9** (3.3 mg, 23%).Fr. 16 was purified by semi-preparative HPLC to give compound **11** (2.0 mg, 1.2%) eluted with MeOH-H_2O-TFA (57:43:0.03, *v/v*). Fr. 21 was subjected to preparative HPLC and eluted with MeOH-H_2O-TFA (60: 40:0.03, *v/v*) to give compound **8** (9.4 mg, 55%).

Structure characterization

21*α*-hydroxy-3-acetyl-11-keto-*β*-boswellic acid (1): Yellow powder (MeOH), UV λ_{max} (MeOH): 254 nm; m.p. 159-161°C. $[\alpha]_D^{22}$+ 1.6 (*c* 0.1, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 1 and 3. HRESIMS *m/z* 609.3014 $[M+2H+Br]^+$ (calcd for $C_{32}H_{48}O_6$, 609.2791).

21*α*-hydroxy-11-keto-*β*-boswellic acid (2): White powder (MeOH), UV λ_{max} (MeOH): 254 nm; m.p. 175-176 °C. $[\alpha]_D^{22}$+3.6 (*c* 0.12, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 1 and 3. HRESIMS *m/z* 567.2916 $[M+2H+Br]^+$ (calcd for $C_{30}H_{46}O_5$, 567.2685).

16*β*,30-dihydroxy-11-keto-*β*-boswellic acid (3): White powder (MeOH), UV λ_{max} (MeOH): 254 nm; m.p. 197-198 °C. $[\alpha]_D^{22}$+ 12.5 (*c* 0.10, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 1 and 3. HRESIMS *m/z* 503.3148 $[M+H]^+$ (calcd for $C_{30}H_{46}O_6$, 503.3373).

15*α*,16*β*-dihydroxy-11-keto-*β*-boswellic acid (4): Yellow crystal (MeOH), UV λ_{max} (MeOH): 254nm;m.p. 191-192 °C. $[\alpha]_D^{22}$ + 7.1 (*c* 0.10, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 1 and 2. HRESIMS *m/z* 503.3313 $[M+H]^+$ (calcd for $C_{30}H_{46}O_6$, 503.3373).

7*β*,21*β*-dihydroxy-11-keto-*β*-boswellic acid (5): White powder (MeOH), UV λ_{max} (MeOH): 254 nm; m.p. 183-184 °C. $[\alpha]_D^{22}$ + 3.8 (*c* 0.10, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 1 and 3. HRESIMS *m/z* 543.3321 $[M-H]^-$ (calcd for $C_{32}H_{48}O_7$, 543.3322).

15*α*,21*β*-dihydroxy-3-acetyl-11-keto-*β*-boswellic acid (6): White powder (MeOH), UV λ_{max} (MeOH): 254 nm; m.p. 183-184 °C. $[\alpha]_D^{22}$ + 3.8 (*c* 0.10, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 1 and 3, respectively; HRESIMS *m/z* 543.3329 $[M-H]^-$ (calcd for $C_{32}H_{48}O_7$, 543.3322).

7*β*,15*α*,21*β*-trihydroxy-3-acetyl-11-keto-*β*-boswellic acid (7): White powder (MeOH), UV λ_{max} (MeOH): 254 nm; m.p. 183-184 °C. $[\alpha]_D^{22}$ + 1.2 (*c* 0.08, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 2 and 3, respectively; HRESIMS *m/z* 559.3274 $[M-H]^-$ (calcd for $C_{32}H_{48}O_8$, 559.3271).

21*β*-hydroxy-11-keto-*β*-boswellic acid (8): colorless crystal (MeOH), UV λ_{max} (MeOH): 254 nm;m.p. 188-189°C. $[\alpha]_D^{22}$ + 4.2 (*c* 0.10, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 2 and 3. HRESIMS *m/z* 487.3328 $[M+H]^+$ (calcd for $C_{30}H_{46}O_5$, 487.3423).

7*β*,21*β*-dihydroxy-11-keto-*β*-boswellic acid (9): colorless crystal (MeOH), UV λ_{max} (MeOH): 254 nm; m.p. 189-190 °C. $[\alpha]_D^{22}$ + 13.7 (*c* 0.08, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 2 and 3. HRESIMS *m/z* 501.3217 $[M-H]^-$ (calcd for $C_{30}H_{46}O_6$, 501.3216).

15*α*,21*β*-dihydroxy-11-keto-*β*-boswellic acid (10): White powder (MeOH), UV λ_{max} (MeOH): 254 nm; m.p. 194-195 °C. $[\alpha]_D^{22}$ + 12.1 (*c* 0.10, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 2 and 3. HRESIMS *m/z* 503.3148 $[M+H]^+$ (calcd for $C_{30}H_{46}O_6$, 503.3373).

3-*epi*-7*β*,21*β*-dihydroxy-11-keto-*β*-boswellic acid (11): Colorless crystal (MeOH), UV λ_{max} (MeOH): 254 nm; m.p. 208-209 °C. $[\alpha]_D^{22}$ + 8.5 (*c* 0.10, MeOH). ^{1}H-NMR (Pry-d_5, 600 MHz) and ^{13}C-NMR (Pry-d_5, 150 MHz) see Tables 2 and 3. HRESIMS *m/z* 503.3313 $[M+H]^+$ (calcd for $C_{30}H_{46}O_6$, 503.3373).

Analysis methods

The samples were analyzed on an Ultimate 3000 HPLC equipped with a DIONEX C-18 column, 4.6 mm×250 mm (5 μm), and diode array detector (DAD). Detection wavelength was set at 254 nm, and the flow rate was 0.8 ml/min.

The mobile phase (for metabolites **2-7**): solvent A (MeOH) and solvent B (0.3% aqueous TFA, *v/v*). A gradient elution program was as follows: initial 0-5 min, using a isocratic elution A-B (30:70, *v/v*); 5-15 min, using a linear change from A-B (30:70, *v/v*) to A-B (60:40, *v/v*); then 15-30 min, using a isocratic elution A-B (60:40, *v/v*); next 30-40 min, sing a linear change from A-B (60:40, *v/v*) to A-B (90:10, *v/v*).

The mobile phase (for metabolite **1**): solvent A (MeOH) and solvent B (0.3% aqueous TFA, *v/v*). A gradient elution program was as follows: initial 0-5 min, using a isocratic elution A-B (30:70, *v/v*); next 5-15 min, using a linear change from A-B (30:70, *v/v*) to A-B (75:25, *v/v*); then 15-20 min, using a linear change from A-B (75:25, *v/v*) to A-B (82:18, *v/v*); next 20-40min, using a isocratic elution A-B (82:18, *v/v*); final 40-45

min, using a linear change from A-B (82:18, *v/v*) to A-B (90:10, *v/v*).

The mobile phase (for metabolites **8-11**): solvent A (MeCN) and solvent B (0.3% aqueous TFA, *v/v*). A gradient elution program was as follows: 0-5 min, using a isocratic elution A-B (10:90, *v/v*); 5-15 min, using a linear change from A-B (10:90, *v/v*) to A-B (33:67, *v/v*); 15-30 min, using a isocratic elution A-B (33:67, *v/v*); 30-40 min, sing a linear change from A-B (33:67, *v/v*) to A-B (70:30, *v/v*); 40-55 min, using a isocratic elution A-B (70:30, *v/v*); 55-65 min, sing a linear change from A-B (30:70, *v/v*) to A-B (10:90, *v/v*).

Inhibitory Effects on NitricOxide Production in LPS-Activated Macrophages

Compounds **1**–**11** were tested for their ability to inhibit LPS-activated nitric oxide production in RAW 264.7 macrophages. This assay was carried out as previously described.[29]

Conclusions

In summary, the biotransformation of two strains filamentous fungi on AKBA and KBA was carried out to obtain eleven metabolites, including seven novel compounds. Both of the enzymatic reactions were mainly included hydroxylation at the various chemical positions. *P. janthinellum* AS 3.510 preferred to catalyze hydroxylation reaction particularly on 21α with the regio- and stereo- selectivity. Moreover, *C. elegans* AS 3.1207 preferred to catalyze hydroxylation reaction particularly on 21β. These biotransformation reactions would be difficult for chemical synthesis. Our results may provide useful information for the further investigation of boswellic acids.

In addition, it should be highlighted that few hydroxylations of ring-E for the skeletons of ursane pentacyclic triterpenoids had been reported. *P. janthinellum* AS 3.510 and *C. elegans* AS 3.1207 realized hydroxylation reaction at ring-E to produce metabolites**1** and **8** with relatively high yielded. Furthermore, Metabolite **1** has the potent anti-inflammatory bioactivity, which could be used as drug or as key building block to prepare the new anti-inflammatory candidate.

Acknowledgements

We thank the National Natural Science Foundation of China (No. 81274047 and 81473334), Education Department of Liaoning Province (LR2014025, L2014352), and Outstanding Youth Science and Technology Talents of Dalian for financial supports.

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