Bioorganic & Medicinal Chemistry Letters 23 (2013) 1338–1342

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Microbial transformation of acetyl-11-keto-β-boswellic acid and their inhibitory activity on LPS-induced NO production

Yan Sun [a,†], Dan Liu [a,b,†], RongGang Xi [c], Xiaobo Wang [c,*], Yan Wang [a], Jie Hou [a], Baojing Zhang [a], Changyuan Wang [a], Kexin Liu [a], Xiaochi Ma [a,*]

[a] College of Pharmacy, Dalian Medical University, Lvshun South Road No. 9, Dalian 116044, China
[b] School of Science, Dalian Nationalities University, Dalian 116600, China
[c] Chinese People's Liberation Army 210 Hospital, Dalian 116021, China

ARTICLE INFO

Article history:
Received 13 October 2012
Revised 15 December 2012
Accepted 27 December 2012
Available online 12 January 2013

Keywords:
Acetyl-11-keto-β-boswellic
Boswellia serrata
Microbial transformation
NO inhibition

ABSTRACT

The capabilities of 20 strains of fungi to transform acetyl-11-keto-β-boswellic (**AKBA**) were screened. And biotransformation of **AKBA** by *Cunninghamella blakesleana* AS 3.970 afforded five metabolites (**1**–**5**), while two metabolites (**6**, **7**) were isolated from biotransformation of *Cunninghamella elegans* AS 3.1207. The chemical structures of these metabolites were identified by spectral methods including 2D NMR and their structures were elucidated as 7β-hydroxy-3-acety-11-keto-β-boswellic acid (**1**), 21β-dihydroxy-3-acety-11-keto-β-boswellic acid (**2**), 7β,22α-dihydroxy-3-acety-11-keto-β-boswellic acid (**3**), 7β,16α-dihydroxy-3-acety-11-keto-β-boswellic acid (**4**), 7β,15α-dihydroxy-3-acety-11-keto-β-boswellic acid (**5**); 7β,15α,21β-trihydroxy-3-acety-11-keto-β-boswellic acid (**6**) and 15α,21β-dihydroxy-3-acety-11-keto-β-boswellic acid (**7**). All these products are previously unknown. Their primary structure–activity relationships (SAR) of inhibition activity on LPS-induced NO production in RAW 264.7 macrophage cells were evaluated.

Boswellia serrata is a type of deciduous tree grows in Africa and Asia.[1] And gum resin extracts of *Boswellia serrata* have been found to represent a promising anti-inflammatory herbal remedy, using as a folk medicine to cure inflammatory and arthritic diseases.[2] Boswellic acids (BAs), as the pentacyclic triterpenes, were the unique and major constituents to *Boswellia* including 11-keto-β-acetyl-11-keto-β-boswellic acid (**AKBA**), boswellic acid (KBA), β-bosewllic acid (BA) and acetyl-β-boswellic acid (ABA).[3] Among these BAs, acetyl-11-keto-β-boswellic acid (**AKBA**) is one of most bioactive compounds with the biological activities involved in anti-inflammatory,[4] anti-arthritic diseases,[5] asthma[6] and peritumoral brain oedema.[7] Compared to NSAIDs, it is associated with better tolerabihty[8] and devoid of typical adverse effects.[9] However, the poor absorption and extensive metabolism limited bioavailability of **AKBA** in clinic.[10] Therefore, it was very necessary to find some new derivatives with enhanced bioactivities and water-solubilities.

Biotransformation is defined as the use of biological systems to produce chemical transformation on synthetic or natural compounds.[11] The catalysts can be enzymes, whole cells (free or immobilized) or microorganisms. It is considered to be a routine economically and ecologically competitive technology for synthetic organic chemists in search of new production routes to obtain the pharmaceutical and agrochemical compounds.[12] The major advantage of biological catalysts is their capability to catalyze novel reactions with regio- and stereo-selectivity.[13] In light of recent developments in chemical industries to develop 'green process', biotransformation route therefore is highly desirable. In recent years, our research work frequently reported to modify the structures of natural products for obtaining new chemical entities, increasing structural diversity and improving physical–chemical properties.[14] Biotransformation has also been employed as an wonderful assistant tool to mimic and predict the mammalian metabolism of biologically active compounds, or to prepare metabolites which are valuable for in vivo metabolism research.[15]

In this study, biotransformation of **AKBA** by *Cunninghamella blakesleana* AS 3.970 and *Cunninghamella elegans* AS 3.1207 were carried out to obtain the various novel derivatives of **AKBA**.[17] In total, five metabolites (**1**–**5**) of **AKBA** by *C. blakesleana* AS 3.970 and two metabolites (**6**, **7**) of **AKBA** from *C. elegans* AS 3.1207 were isolated[18] and identified by the extensive spectral methods including 2D NMR (HMQC, HMBC, ^{1}H–^{1}H-COSY and NOESY) and HR-MS, and all metabolites of **AKBA** are novel (Fig. 1). In addition, their inhibitory activity on LPS-induced NO production in RAW 264.7 macrophage cells were also investigated.

* Corresponding authors. Tel./fax: +86 411 86110419.
E-mail addresses: wxbbenson0653@sina.com (X. Wang), maxc1978@163.com (X. Ma).
† These two authors contributed equally to this work.

0960-894X/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmcl.2012.12.086

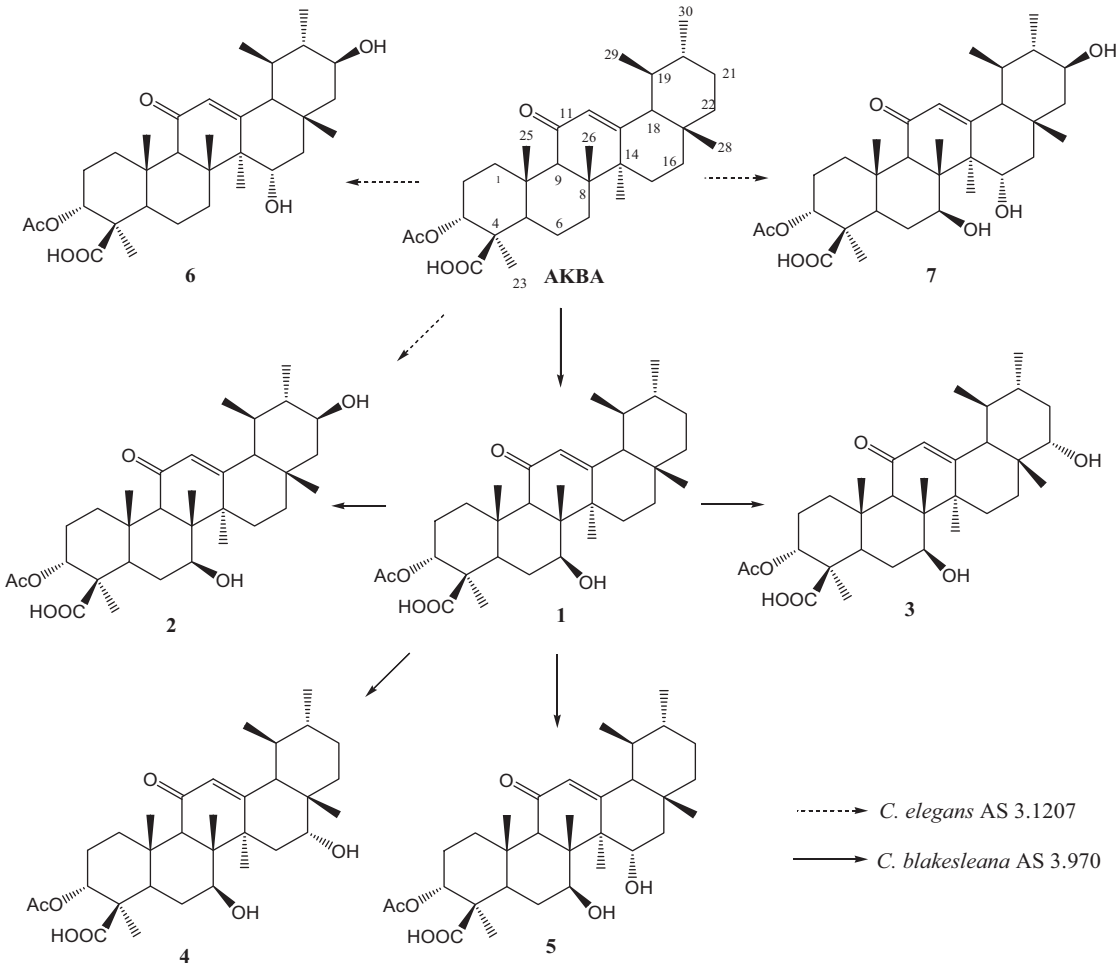

Figure 1. A proposed biotransformation pathway of **AKBA** by *C. blakesleana* AS 3.970 and *C. elegans* AS 3.1207.

The molecular formula of **1** was deduced to be $C_{32}H_{48}O_6$ [19a] using HR-ESIMS ([M−H]$^-$ *m*/*z* 527.3378). In its ^{13}C NMR spectrum, a new oxygen-bearing methine carbon at δ 72.8 was observed. In the HMBC spectrum, the long-range correlations of carbon signal at δ 72.8 with Me-26 (δ 1.56), H-9 (δ 2.77), H-6a (δ 2.55), H-6b (δ 2.70) and H-5 (δ 1.94), indicated that a hydroxyl group was introduced at C-7 in the molecular of **1**. In NOESY spectrum, a NOE enhancement between H-7 (δ 4.47) and Me-27 (δ 1.52), suggested β-configuration for HO-7. These data confirmed the chemical structure of **1** as 7β-hydroxy-3-acety-11-keto-β-boswellic acid.

Compound **2** was white powder in MeOH. Its HR-ESIMS provided molecular formula of $C_{32}H_{48}O_7$[19b] 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 4.45 and δ 3.68. The ^{13}C NMR spectrum showed two additional carbon signals at δ 72.9 and δ 70.3. In HMBC spectrum, the long-range correlations of δ 70.3 with Me-30 (δ 1.30), H-22a (δ 1.54) and H-22b (δ 2.04), confirmed the hydroxyl group should be located at C-21 position. In ^{1}H–^{1}H-COSY spectrum, the proton of δ 4.45 had the correlations with H-6a (δ 2.78), H-6b (δ 2.53) and H-9 (δ 2.74) suggesting compound **2** possessed 7-hydroxyl group. In NOESY spectrum, the NOE enhancements of H-7 (δ 4.45) with Me-27 (δ 1.52) indicated that HO-7 should be in β-orientation. Meantime, the NOE enhancement of H-21 (δ 3.68) with H-19 (δ 1.70) exhibited the β-orientation of HO-21. Therefore, compound **2** was identified as 7β,21β-dihydroxy-3-acety-11-keto-β-boswellic acid.

Compound **3** exhibited a *quasi*-molecular ion peak at *m*/*z* 543.3324 [M−H]$^-$ in the HR-ESIMS, corresponding to its molecular formula of $C_{32}H_{48}O_7$.[19c] Its ^{1}H NMR spectrum were similar to that of **AKBA**, but two new oxygen-bearing methine protons at δ 4.51 and δ 3.60 were observed, which were directly correlated with the carbon signals at δ 72.7 and δ 74.2, respectively, in HMQC experiment. The HMBC correlations of H-7 (δ 4.51) with Me-26 (δ 12.6) and C-14 (δ 46.4) were observed, while H-22 (δ 3.60) showed the cross peaks with C-18 (δ 55.7) and C-20 (δ 32.2). These evidences confirmed that two hydroxyl groups were substituted at C-7 and C-22. The β-configuration of HO-7 was established according to the NOE enhancement between H-7 (δ 4.51) and Me-27 (δ 1.59), while NOE correlation of H-22 (δ 3.60) with Me-28 (δ 1.24) were also observed, all of which indicating that HO-22 should be in an α-orientation. On the basis of above analysis, the structure of **3** was determined as 7β,22α-dihydroxy-3-acety-11-keto-β-boswellic acid.

The molecular formula $C_{32}H_{48}O_7$ [19d] of **4** was deduced from HR-ESIMS [M−H]$^-$ *m*/*z* 543.3323, which indicating that it was a dihydroxylated derivative of **AKBA**. Its ^{13}C NMR spectrum exhibited two additional oxygen-bearing methine signals at δ 72.8 and δ 77.5, and its ^{1}H NMR spectrum showed two new protons at δ 4.51 (dd, *J* = 4.2, 11.4 Hz) and δ 3.58 (dd, *J* = 3.6, 11.4 Hz). In HMBC spectrum, H-7 (δ 4.51) had the long-range correlations with Me-26 (δ 12.6) and C-14 (δ 46.7), which suggesting that compound **4** possessed 7-hydroxyl group. In addition, the proton signal of δ 3.58 had the HMBC correlation of C-22 (δ 21.5) and its corresponding carbon of δ 77.5 had the long-range correlations with H-21a (δ 1.72), H-21b (δ 1.89) and Me-28 (δ 1.26), suggesting a hydroxyl group was substituted at C-16. In NOESY spectrum, the NOE