Phytochemistry 72 (2011) 1779–1785

Contents lists available at ScienceDirect

Phytochemistry

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

Biotransformation of bufadienolides by cell suspension cultures of *Saussurea involucrata*

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ARTICLE INFO

Article history:
Received 17 September 2010
Received in revised form 4 January 2011
Available online 1 June 2011

Keywords:
Saussurea involucrata
Asterates
Biotransformation
Bufadienolides
Cell suspension culture
Cytotoxicity

ABSTRACT

The biotransformation of three bioactive bufadienolides, namely, bufotalin (**1**), telocinobufagin (**2**), and gamabufotalin (**3**) by cell suspension cultures of *Saussurea involucrata* yielded 11 products. Bufotalin yielded 3-*epi*-bufotalin (**1a**), 3-*epi*-desacetylbufotalin (**1b**), 3-*epi*-bufotalin 3-*O*-β-D-glucoside (**1c**), 1β-hydroxybufotalin (**1d**), and 5β-hydroxybufotalin (**1e**); telocinobufagin yielded 3-dehydroscillarenin (**2a**), 3-dehydrobufalin (**2b**), and 3-*epi*-telocinobufagin (**2c**); and gamabufotalin yielded 3-*epi*-gamabufotalin (**3a**), 3-dehydrogamabufotalin (**3b**), and 3-dehydro-Δ^1-gamabufotalin (**3c**), respectively. Among these 11 products, **1a**, **1b**, **1c**, **1d**, **3a** and **3c** are previously unreported. The structures of these metabolites were elucidated based on NMR spectroscopic analyses and mass spectrometry. Most metabolites showed significant cytotoxic activities against human hepatoma (HepG2) and breast cancer (MCF-7) cell lines. In addition, the time course for the biotransformation of **3** was investigated.

1. Introduction

Bufadienolides, a class of C-24 steroids, are the major bioactive constituents of the traditional Chinese medicine Chan'Su (Venenum Bufonis). This type of cardiotonic steroids display a range of pharmacological activities including positive inotropic, antiviral, antiangiogenic and anti-inflammatory activities (Inada et al., 1999; Cunha Filho et al., 2010; Yu et al., 2008). Recently, many studies have focused on their significant cytotoxicities against a variety of cancer cells, such as human prostate, lung, astrocytoma and gastric tumor cell lines (Wang et al., 2009; Li et al., 2010; Cunha Filho et al., 2010). More interestingly, recent studies established that certain chemically modified, non-cardioactive, bufadienolides could kill human cancer cells while sparing normal cells (Daniel et al., 2003). The increased interest in bufadienolides prompted us to synthesize new derivatives that could be anti-tumor lead compounds, or could help establish structure–activity and structure–toxicity relationship (Hilton et al., 2010; Kamano et al., 2002), thus providing reliable information for future drug design.

Biotransformation, a process whereby substrates are altered by the action of enzymes or microorganisms, is an important method for increasing the structural diversity of bufadienolides (Ye et al., 2002b, 2004a, 2005a; Ye and Guo, 2005; Zhang et al., 2007; Zhao et al., 2007). Compared to chemical means, biocatalysis does not require steps for protecting labile functional groups (Simeo and Sinisterra, 2009). In addition it has advantages of high stereoselectivity and regioselectivity. According to previous reports, some biotransformed products from bufadienolides showed even more potent activity than their precursors against cancer cells (Ye et al., 2004b, 2005b; Cunha Filho et al., 2010). Bufotalin, telocinobufagin and gamabufotalin are three cytotoxic bufadienolides isolated from Chan'Su. Their biotransformation by plant cells has not been pursued thus far. In this study, the biotransformation of these three bufadienolides by cultured suspension cells of *Saussurea involucrata* is reported. Cytotoxic activities of the metabolites against HepG2 human hepatoma and MCF-7 human breast cancer cells were also evaluated.

2. Results and discussion

The substrates bufotalin (**1**), telocinobufagin (**2**) and gamabufotalin (**3**) were isolated from the traditional Chinese medicine Chan'Su. Fermentation of these compounds with *S. involucrata* for seven days resulted in five known and six previously unreported metabolites (Figs. 1–3). The five known metabolites were identified as 5β-hydroxybufotalin (**1e**) (Kamano et al., 1979), 3-dehydroscillarenin (**2a**) (Kamano and Pettit, 1974), 3-dehydrobufalin

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doi:10.1016/j.phytochem.2011.05.004

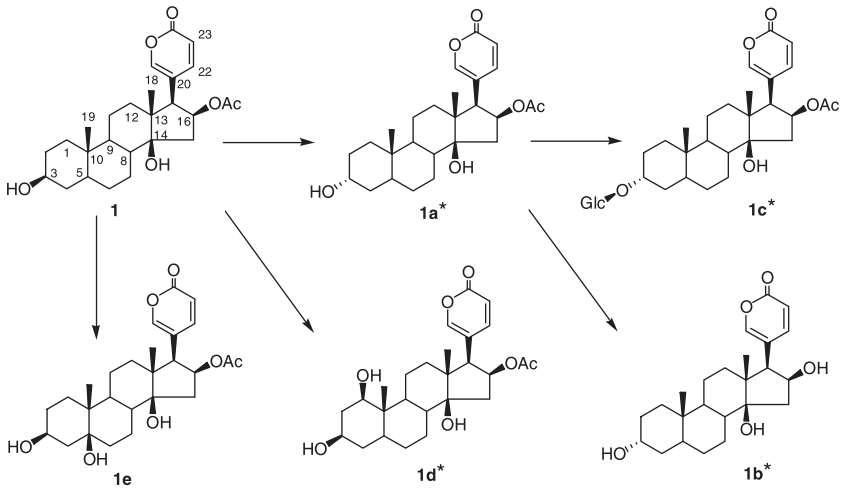

Fig. 1. Biotransformation products of **1** by *S. involucrata* cultured cells (* previously unreported compound).

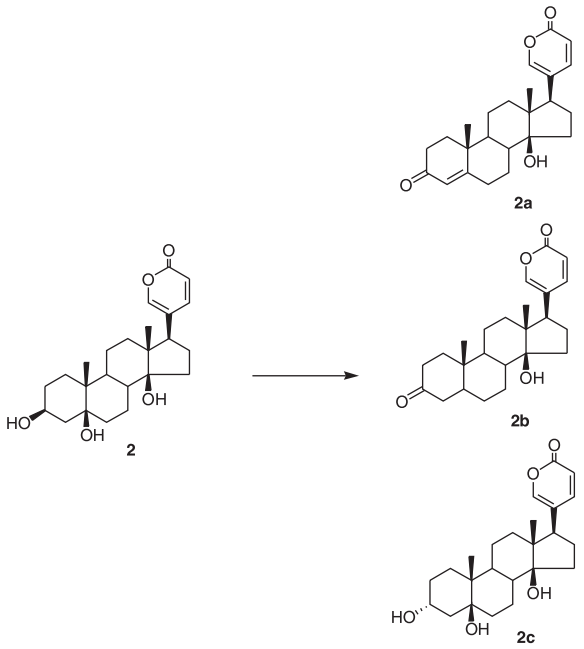

Fig. 2. Biotransformation products of **2** by *S. involucrata* cultured cells.

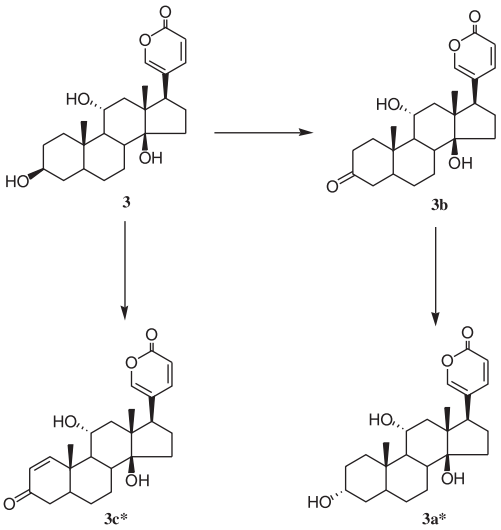

Fig. 3. A proposed biotransformation pathway of gamabufotalin (**3**) by *S. involucrata* cultured cells (* previously unreported compound).

(**2b**) (Ma et al., 2007b), 3-*epi*-telocinobufagin (**2c**) (Ma et al., 2007a), and 3-dehydrogamabufotalin (**3b**) (Linde and Loehrer, 1991) by comparing their spectroscopic data with those in the literature. The structures of the six other metabolites, namely, 3-*epi*-bufotalin (**1a**), 3-*epi*-desacetylbufotalin (**1b**), 3-*epi*-bufotalin 3-*O*-β-D-glucoside (**1c**), 1β-hydroxybufotalin (**1d**), 3-*epi*-gamabufotalin (**3a**), and 3-dehydro-Δ^1-gamabufotalin (**3c**) were elucidated through mass spectrometry, as well as ^{1}H NMR, ^{13}C NMR, and 2D NMR spectroscopic analyses. ^{1}H and ^{13}C NMR signals of the compounds were fully assigned based on HSQC, HMBC, ^{1}H–^{1}H COSY and NOESY spectra, as shown in Tables 1 and 2.

2.1. Structure elucidation of compounds

Compound **1a** was obtained as a white power. Its molecular formula of $C_{26}H_{36}O_6$ was established from HREIMS ([M]$^+$ at *m*/*z* 444.2495, calcd 444.2512). Detailed analysis of its 1D and 2D NMR spectra indicated that the planar structure of **1a** was identical to that of **1**. The ^{1}H and ^{13}C NMR spectroscopic data of **1** and **1a** were very similar except for the ring A signals. The resonance of H-3 (δ_H 3.40) appeared as a broad multiplet ($W_{1/2}$ 20 Hz), indicating that the 3-OH group of **1a** should be located in the α-position (Ye et al., 2002a). NOE enhancements between H-3 and H-4β (δ_H 0.91), H-3 and H-5 (δ_H 1.28) further confirmed this assignment. The ring A signals of **1a** were in good agreement with those of previously reported 3α-hydroxyl bufadienolides (Ye et al., 2003). Thus, the structure of **1a** was assigned as 3-*epi*-bufotalin.

Compound **1b** was isolated as a white powder with molecular formula of $C_{24}H_{34}O_5$ based on HREIMS. The absence of resonance corresponding to the acetyl group indicated that **1b** could be a deacetylated derivative of **1**. Accordingly, the C-16 resonated at a significantly higher field (δ_C 70.9, v.s. δ_C 74.1 for **1**). The 3-OH of **1b** was deduced to be in the α-configuration by comparing its ^{1}H NMR spectrum with that of **1a**. The NOESY spectrum of **1b** confirmed the assignment. On the basis of the above analysis, compound **1b** was identified as 3-*epi*-desacetylbufotalin. All the ^{1}H and ^{13}C NMR spectroscopic data were unambiguously assigned by 2D NMR spectra.

Compound **1c** possessed a molecular formula of $C_{32}H_{46}O_{11}$, determined by HRESIMS, which was 162 mass units greater than substrate **1**, implying one glucosyl residue in **1c**. Detailed analysis