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Biotransformation of sesquiterpenoids having α,β-unsaturated carbonyl groups with cultured plant cells of *Marchantia polymorpha*

Mohamed-Elamir F. Hegazy [a], Chika Kuwata [a], Akihito Matsushima [b], Ahmed A. Ahmed [c], Toshifumi Hirata [a,*]

[a] *Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan*
[b] *Natural Science Center for Basic Research and Development, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan*
[c] *Department of Chemistry, Faculty of Science, El-Minia University, El-Minia 61519, Egypt*

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Abstract

The biotransformation of sesquiterpenoids having an α,β-unsaturated carbonyl group, such as α-santonin (**1**), lancerodiol *p*-hydroxybenzoate (**2**), 8,9-dehydronootkatone (**3**), and nootkatone (**4**), with cultured suspension cells of *Marchantia polymorpha* was investigated. It was found that the C─C double bond of **1** and **2** was hydrogenated to give 1,2-dihydro-α-santonin (**5**) and 3,4-dihydrolancerodiol *p*-hydroxybenzoate (**6**), respectively, while the allylic position of the C─C double bond of **3** and **4** was hydroxylated to give 13-hydroxy-8,9-dehydronootkatone (**7**) and 9-hydroxynootkatone (**8**), respectively.

Keywords: Biotransformation; *Marchantia polymorpha*; Hydrogenation; Hydroxylation; α-Santonin; Lancerodiol *p*-hydroxybenzoate; 8,9-Dehydronootkatone; Nootkatone

1. Introduction

Terpenoids are a large class of naturally occurring compounds, and are not only known as raw materials for flavor and fragrances but also biologically active substances against microorganisms, plants, insects, and animals. Since a great majority of biologically active terpenoids are produced as plant secondary metabolites, many naturally occurring terpenoids were modified with biocatalysts to get substances with an enhanced biological activity [1–13].

The ability of biocatalysts to convert foreign substrates into chemo-, regio-, stereo-, and enantioselectively useful substances under mild condition is one of great interest, as products may be formed which are difficult to prepare by synthetic chemical methods. Recently, we found that cultured plant cells contain several different enzymes participating in the asymmetric hydrogenation of the C–C double bond of monoterpene enones [14,15]. Recently, it was found that cultured cells of *Marchantia polymorpha* have larger potentiality to hydrogenate the C–C double bond of maleimides in comparison to other kinds of cultured plant cells [16]. In order to develop useful biocatalysts, we have further investigated the potentiality of these plant cells to convert natural sesquiterpenoids having more complex enone structures, such as α-santonin (**1**) [2,8,11], lancerodiol *p*-hydroxybenzoate (**2**) [17,18], 8,9-dehydronootkatone (**3**), and nootkatone (**4**) [12], as model compounds with cultured suspension cells of *M. polymorpha*.

* Corresponding author. Tel.: +81 82 424 7435; fax: +81 82 424 7435.
E-mail address: thirata@sci.hiroshima-u.ac.jp (T. Hirata).

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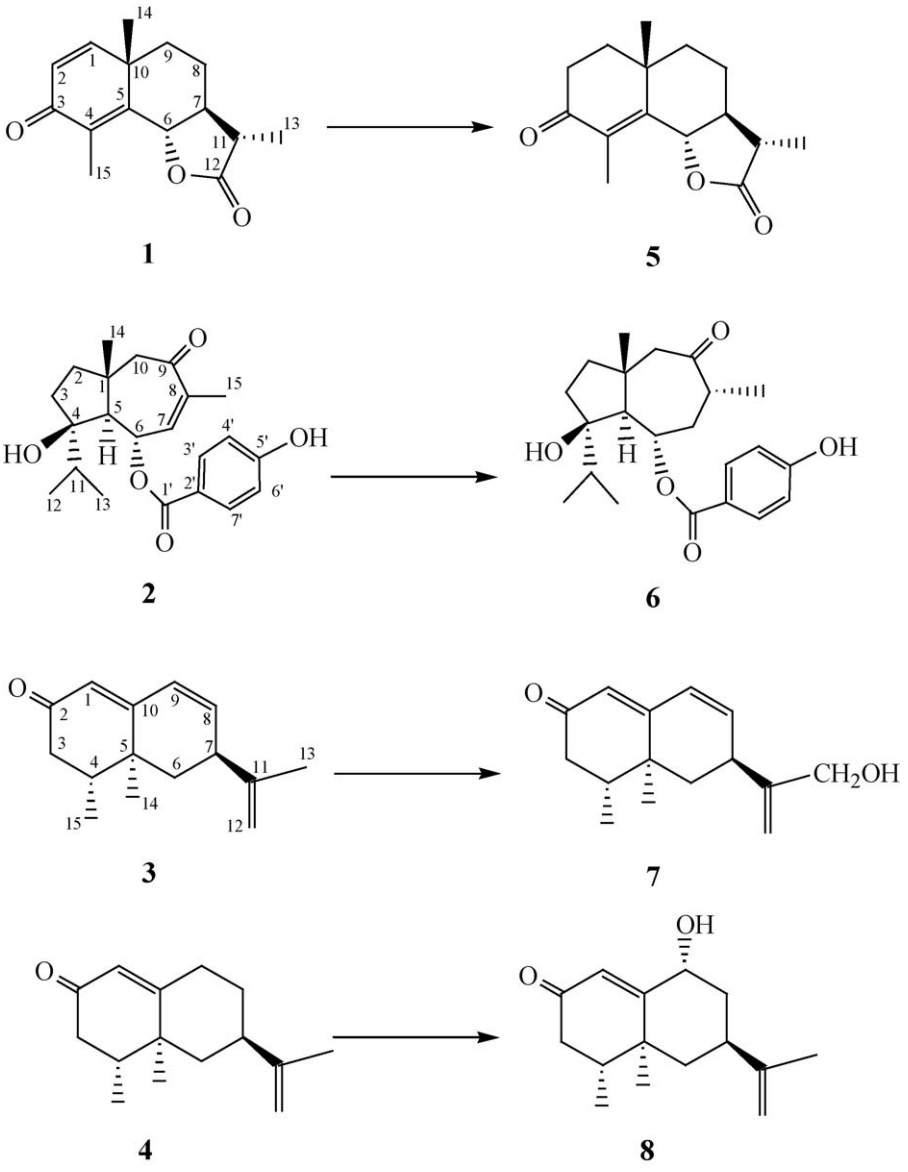

2. Experimental

2.1. Analysis

Analytical and preparative TLCs were carried out on glass sheets (0.25 and 0.5 mm) coated with silica gel (Merck silica gel 60; GF_{254}). GLC was carried out with FID and a capillary column (0.25 mm × 30 m) coated with 0.25 μm ZB-5 (Zebron) using N_2 as carrier gas (60 cm^3 min^{-1}) at column temperature 100–250 °C. HPLC was carried out on a Puresil C_{18} column (Waters) using CH_3CN:H_2O = 2:3 (v/v) as the eluent. ^1H and ^{13}C NMR spectra were obtained using a JEOL LA500 spectrometer using tetramethylsilane as an internal standard. Mass spectra were performed using a JEOL SX-102A spectrometer with an ionizing energy of 70 eV.

2.2. Substrates

Substrates used for biotransformation experiments were α-santonin (Aldrich), lancerodiol *p*-hydroxybenzoate (isolated from *Ferula sinaica* species, Asteraceae), 8,9-dehydronootkatone (donated from Prof. Y. Noma of Tokushima Bunri University) and nootkatone (Aldrich).

2.3. Plant material

The cells of *M. polymorpha* [19] have been subcultured routinely every 3 weeks using MSK-II medium [20], containing 2% glucose, 0.1% inositol, 10 mM of 2,4-dichlorophenoxyacetic acid (2,4-D), more than 10 years in our laboratory. Prior to use for biotransformation experiments, the cultured cells were transplanted to 300 ml conical flask containing 100 ml of MSK-II