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RESEARCH ARTICLE



Fungal biotransformation of cedramber

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ABSTRACT

Cedramber (cedrol methyl ether) (**1**) is a fragrance compound used in cosmetics such as soap, detergents, creams, lotions and perfumes. New cedrol derivatives are of interest in this context. Here we report the first biotransformation reaction of cedramber by *Aspergillus niger* ATCC 10549. The conversion afforded two known cedrol derivatives namely, 12 β -hydroxy cedrol (**2**), and 3 β -hydroxy cedrol (**3**), in 11.9, and 15.5% yields, respectively. The new metabolite chemical structures were characterized by spectroscopic techniques such as FT-IR, GC/MS and NMR analyses.

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Cedramber; fragrance; microbial transformation

Introduction

Fragrance compounds are used in daily life as well as in diverse industries such as cosmetics and toiletries (Rowe 2005; Longo and Sanroman 2006). They contain many unique structures with various functional groups, allowing a wide variety of odours ranging from fruity flavours to floral notes (Carroll et al. 2016; Pelosi 2016). A variety of fragrance compounds also serve as pheromones, natural insecticides, etc., and are used as precursor molecules for the synthesis of certain natural products (Berger 2007; Gershenzon and Dudareva 2007).

Fragrances are highly demanded fine chemicals, and their conventional productions are based on chemical synthesis or extraction from natural sources. Isolation of fragrance compounds from plants is an expensive process due to their complex protocols. Although chemical syntheses serve as a good alternative, the consumer's preference is rather for natural products (Winterhalter and Schreier 1993; Seitz 1994; Welsh 1994; Soares et al. 2000).

During the past few years, worldwide increasing market demand for safe additives, natural product-based materials gained more attention for the development of new eco-friendly industrial processes for their production (Singh et al. 2019). The utilization of biotechnological processes, especially microbial

transformation is accepted as a natural way, on the production of fragrance compounds are among the production options in this context.

Sesquiterpenes are among fragrance compounds, which are subjected to evaluation for the production of their new derivatives using biotransformation reactions in recent years. One of the important representatives “cedrol”, and its derivatives were previously biotransformed by different organisms. As a result, mono-, di-hydroxylated and some poly-hydroxylated metabolites at different positions were reported (Abraham et al. 1987; Hanson and Nasir 1993; Maatooq et al. 1993; Fraga et al. 1996; Kiran et al. 2010; Sultan et al. 2013). Such derivatives are relatively important, not only as food/cosmetic preservatives especially in perfumery and fragrances but also due to their diverse biological activities such as α -glucosidase and β -secretase inhibitors (Miyazawa et al. 2003; Marumoto et al. 2017).

Cedramber (**1**), also recognized as cedrol methyl ether, is an oxygenated sesquiterpene synthesized from cedrol by methylation. It is used as a woody spicy fragrance in soap, detergents, creams, lotions and perfumes (Arctander 1969). The aim of this study was to carry out biotransformation of cedramber with a variety of fungi to produce new derivatives as potential fragrances. To the best of our knowledge,

this is the first report on the microbial transformation of cedramber.

Materials and methods

General experimental procedures

Cedramber (>95%) was kindly provided by IFF Co., USA. High purity chemicals and solvents obtained from commercial sources (Sigma-Aldrich and Merck) were used. Melting point data were determined on a Gallenkamp MPD350 melting point apparatus and are uncorrected. FT-IR spectra were measured using KBr pellets. The MS was recorded on a Water SYNAPT G1 MS system VG whereas the GC/MS analyses were carried out with a Shimadzu QC 2010 system using CPSil-5CB (25 m × 0.25 mm, 0.25 µm film thickness) and Agilent 5975 GC-MSD system using Innnowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness). Helium was used as carrier gas (0.8 mL/min) and GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min. It was then kept constant at 220 °C for 10 min and programmed to 240 °C at a rate of 1 °C/min. The split ratio was adjusted at 40:1 and the injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV with a mass range from m/z 35 to 450. ^1H NMR and ^{13}C NMR spectra were determined by a Varian Mercury Plus 400 spectrometer, while the DEPT spectra were measured at 90 MHz in deuteriochloroform with tetramethylsilane as an internal standard reference. Silica gel-type 60 (Merck, 230–400 mesh) was used for column chromatography. TLC was carried out on a 0.25 mm thick silica gel plate (Merck silica gel 60 GF₂₅₄) in petroleum ether/ethyl acetate (1:1, v:v). Compounds were detected either under UV light (at 254 nm and 366 nm) or visualized by reacting with anisaldehyde (0.5 mL), methanol (85 mL), sulphuric acid (8 mL), and acetic acid (10 mL) after heating.

Microorganisms

Fungal cultures of this study were obtained from the USA Agriculture Research Service Culture Collection (NRRL), American Type Culture Collection (ATCC), and the culture collection of the Department of Pharmacognosy at the Faculty of Pharmacy at Anadolu University, Eskişehir, Turkey. All microorganisms were stored at –85 °C (Ultrafreezer, New Brunswick) in 15% glycerol and maintained on nutrient agar (Merck) and malt extract agar (Merck) slants at 4 °C, respectively. Prior to use, strains were sub-

cultured twice on nutrient agar and malt extract agar to check the purity of microorganisms.

Biotransformation strains

Fungi used in the biotransformation reactions are as follows: *Aspergillus parasiticus* NRRL 2999, *Aspergillus niger* NRRL 326, *Aspergillus niger* ATCC 10549, *Aspergillus alliaceus* NRRL 317, *Penicillium claviforme* MR 376, *Penicillium adametzii* NRRL 737, *Penicillium chrysogenum* NRRL 792, *Fusarium solani* ATCC 1284, *Fusarium moniliforme* NRRL 2374, *Fusarium culmorum* (isolate), *Saccharomyces cerevisiae* ATCC 9763, *Hansenula anomala* ATCC 20170, *Sporobolomyces roseus* ATCC 11385, *Alternaria alternata* NRRL 20593, *Mucor ramannianus* ATCC 1839, *Neurospora crassa* (wild type), *Corynespora cassicola* DSM 62475, *Trametes versicolour* ATCC 200801 and *Phanerochaete chrysosporium* ME 446.

Conditions of cultivation and transformation

The cultures were maintained and precultured on potato dextrose agar (PDA) slants at 5 °C and 25 °C, respectively. The slants were prepared as described in our previous study (Kıran et al. 2004). α -Medium used in biotransformation experiments with *A. niger* ATCC 10549 was prepared by mixing glucose (15 g), potassium dihydrogen phosphate (1 g), saccharose (15 g), polypeptone (5 g), potassium chloride (500 mg), magnesium sulphate (500 mg) and iron (II) sulphate (10 mg) in distilled water (1 L). Cedramber (500 mg) was added after 2 days of growth and the fermentations were continued for a further 7 days. The mycelium was filtered off and the broth was acidified with dilute HCl and extracted with ethyl acetate. The extract was dried and the solvent evaporated to give a residue chromatographed on silica with increasing concentrations of ethyl acetate in light petroleum or *n*-hexane (Kıran et al. 2018).

Biotransformation of cedramber by *A. niger*

Incubation of cedramber **1** with *A. niger* ATCC 10549 for 7 days two metabolites **2–3** were isolated (Figure 1). Elution with ethyl acetate in petroleum ether (35:65) afforded 12 β -hydroxycedrol **2** (60 mg, 11.9%); mp 117–119 °C, lit. (Hanson and Nasir 1993); mp 120 °C; IR ν_{max} 3285 cm^{–1}, MS: m/z 238 [M]⁺; ^1H NMR (CDCl₃): 1.02 (3H, s, 14-CH₃), 1.27 (3H, s, 15-CH₃), 1.33 (3H, s, 13-CH₃), 3.46 (1H, dd, J = 7.7 and 10.4,

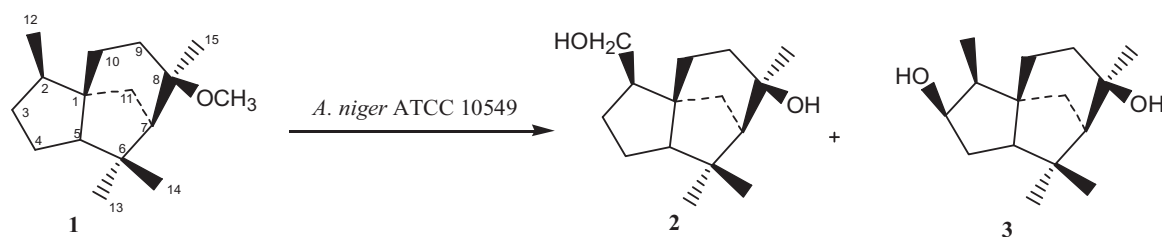


Figure 1. Cedramber (1) biotransformation and its metabolites 2, 3.

Table 1. ^{13}C NMR data for cedramber and its metabolites.

C atom	1	2	3
1	54.0	53.2	52.6
2	41.5	50.3	46.0
3	37.0	32.1	79.8
4	26.7	25.8	32.7
5	56.8	57.4	54.3
6	43.2	43.0	42.9
7	55.4	61.1	61.4
8	78.4	75.0	74.9
9	33.1	34.9	35.0
10	31.3	30.9	33.9
11	41.2	42.6	42.4
12	15.6	63.7	9.7
13	28.8	27.5	27.5
14	25.3	29.0	29.1
15	24.3	30.2	30.2
16	48.1		

12 β -CH₂), 3.65 (1H, dd, J =6.9 and 10.4, 12 β -CH₂); ^{13}C NMR: see Table 1, lit (Abraham et al. 1987).

Elution with ethyl acetate in petroleum ether (30:70) afforded 3 β -hydroxycedrol **3** (78 mg, 15.5%); mp 145–147 °C, lit (Hanson and Nasir 1993); mp 150 °C; IR V_{max} 3285 cm⁻¹, MS: m/z 238 [M]⁺; ^1H NMR (CDCl₃): 0.9 (3H, d, J =7.1, 12-CH₃), 1.0 (3H, s, 13-CH₃), 1.26 (3H, s, 15-CH₃), 1.34 (3H, s, 14-CH₃), 4.28 (1H, dd, J =3.8 and 8.0, 3 α -CH); ^{13}C NMR: see Table 1, lit. (Abraham et al. 1987; Collins and Reese 2001; Miyazawa et al. 2003).

Results and discussion

Cedramber biotransformation screening experiments were carried out using 19 fungi. In general, the resulting complex metabolite mixtures could not be separated by column chromatography apart from *Aspergillus niger* ATCC 10549.

Microbial transformation of cedramber (**1**) with *A. niger* for 7 days afforded compounds **2** and **3**, respectively as illustrated in Figure 1. Their structures were elucidated as follows: The MS of the first metabolite showed a molecular ion at m/z 238 consistent with the formula of C₁₅H₂₆O₂. The FT-IR spectrum showed broad hydroxyl absorption at 3285 cm⁻¹. The ^1H NMR spectrum of **2** indicated the appearance of new hydroxyl-bearing methylene protons at δ_{H} 3.46 (1H, dd, J =7.7 and 10.4), and at 3.65 (1H, dd, J =6.9 and

10.4 MHz) ppm, when compared to **1**, suggesting two hydroxyl groups. Two signals at δ_{C} 15.6 ppm were assigned to a methyl carbon (possibly C-12) and 48.1 ppm attributed to the methoxy signal at C-16 in cedramber, which was not present in the ^{13}C NMR spectrum of the metabolite. In addition, compound **2** indicated signals for overall 15 carbon atoms, while the DEPT spectra indicated the presence of three methyl, five methylene, one hydroxy-bearing methylene, three methine and quaternary carbon signals, respectively. The resulting data suggested that a new hydroxyl group was introduced at one of the methyl groups (C-12, C-13, C-14 or C-15), where the methoxy group at C-8 was demethylated. The metabolite was assigned as 12 β -hydroxy cedrol (**2**) by comparing its spectroscopic data with α -cedrol and its known metabolites reported (Abraham et al. 1987; Hanson and Nasir 1993). Hence, the same compound was reported as a biotransformation metabolite previously from *Glomerella cingulata* (Miyazawa et al. 1995) and *Neurospora crassa* (Kiran et al. 2010).

The metabolite **3** highlighted a molecular ion peak at m/z 238 according to the MS data, corresponding to the molecular formula of C₁₅H₂₆O₂. A broad strong absorption at 3285 cm⁻¹ was observed in the FT-IR spectrum. The ^1H NMR spectrum of metabolite **3** indicated an additional hydroxyl-bearing methylene proton (2H, dd) at δ_{H} 4.28 ppm when compared to **1**. This was further confirmed by the ^{13}C NMR spectrum, which showed a hydroxy-bearing methylene signal at δ_{C} 79.8 ppm, respectively. The downfield shifts of the signal at δ_{C} 9.7 ppm assigned to C-12 in **1** suggested that the hydroxyl group was located at position C-3. In addition, ^1H -NMR spectrum of metabolite **3** also lacked a methoxy signal at δ_{C} 48.1 ppm, in the cedramber structure, indicating that the methoxy group at C-8 was demethylated. Based on these spectroscopic analyses, the metabolite **3** was characterized as 3 β -hydroxy cedrol, which was previously reported as a biotransformation product by *Cephalosporium aphidicola* (Hanson and Nasir 1993), *Glomerella cingulata* (Miyazawa et al. 1995) and *Neurospora crassa* (Kiran et al. 2010).

In conclusion, the present transformations were superior in the timeframe and yield when compared to previous studies (Miyazawa et al. 1995; Kiran et al. 2010). Unfortunately, neither mechanistic nor optimization studies were conducted in the present report, which will be worth doing in future experimental studies.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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