BIOTRANSFORMATIONS OF (-)-MYRTENOL AND (-)-NOPOL BY *ASPERGILLUS TAMARII* MRC 72400

Semra YILMAZER KESKİN[1*], Kudret YILDIRIM[1], Cavit UYANIK[2]

[1]Department of Chemistry, Faculty of Science and Art, University of Sakarya, Sakarya, Turkey
[2]Department of Chemistry, Faculty of Science and Art, University of Kocaeli, İzmit, Turkey
syilmazer@sakarya.edu.tr

ABSTRACT

The biotransformation of (-)-myrtenol (1) and (-)-nopol (2) by *Aspergillus tamarii* MRC 72400 was described. The biotransformation of (-)-myrtenol (1) with *A. tamarii* for 7 days afforded (-)-p-menth-1-en-7,8-diol (3). The biotransformation of (-)-nopol (2) by *A. tamarii* for 7 days afforded (-)-7-hydroxymethyl-1-p-menthen-8-ol (4).

Key words: (-)-Myrtenol, (-)-Nopol, *Aspergillus tamarii*, Biotransformation

(-)-MİRTENOL VE (-)-NOPOL'ÜN *ASPERGİLLUS TAMARİİ* MRC 72400 İLE BİYOTRANSFORMASYONLARI

ÖZET

(-)-Mirtenol (1) and (-)-nopol (2)'ün *Aspergillus tamarii* MRC 72400 ile biyotransformasyonları gerçekleştirildi. (-)-Mirtenol (1)'ün *A. tamarii* ile 7 gün süren inkübasyonu neticesinde (-)-p-ment-1-en-7,8-diol (3) elde edildi. *A. tamarii* ile (-)-nopol (2)'ün 7 gün süren inkübasyonunda (-)-7-hidroksimetil-1-p-menten-8-ol (4) bileşiği elde edildi.

Anahtar Kelimeler: (-)-Mirtenol, (-)-Nopol, *Aspergillus tamarii*, Biyotransformasyon

1. INTRODUCTION

(-)-Myrtenol (**1**) and (-)-nopol (**2**) are structurally related monoterpenoids. (-)-Myrtenol is a natural **primary** alcohol used in household cleaners, detergents, fine fragrances, shampoos, toilet soaps and other toiletries [1]. (-)-Nopol is a synthetic primary alcohol used in the synthesis of pesticides and as a fragrance material in the manufacture of soaps, detergents, polishes and other household products [2].

Microbial and enzymatic biotransformations of readily available monoterpenoids into more valuable compounds have drawn attention of many scientists due to their economical potential to perfume, food and pharmaceutical industries [3].

(-)-Myrtenol and (-)-nopol have been used as substrates in some biotransformations. Incubation of (-)-myrtenol with *Picea abies* suspension culture afforded (-)-myrtenal and (-)-myrtanol [4]. Incubation of (-)-nopol with *Glomerella cingulata*, afforded (-)-4-hydroxynopol, 4-oxonopol and 5-hydroxynopol [7]. Incubation of (-)-nopol by *Cephalosporium aphidicola* afforded (-)-4β-methoxynopol and (-)-4β-hydroxynopo [5]. Its incubation with *Aspergillus niger* afforded 7-hydroxymethyl-1-*p*-menthen-8-ol [6]. The dark brown mould *Aspergillus tamarii* has been especially used for steroid biotransformations and given interesting

results such as steroidal D lactones' production via ring D oxidation and steroidal side chain cleavage [8]. In this work, the biotransformations of (-)-myrtenol and (-)-nopol by *Aspergillus tamarii* MRC 72400 were presented.

2. EXPERIMENTS

(-)-Myrtenol and (-)-nopol were purchased from Sigma-Aldrich. Solvents were of analytical grade and were purchased from Merck. The ingredients for liquid medium were also purchased from Merck. The metabolites were purified by column chromatography on silica gel 60 (230-400 mesh, Merck 107734) and were eluted with increasing concentrations of ethyl acetate in hexane. 0.2 mm thick Merck Kieselgel 60 F254 TLC plates were used to check the purity and the spots were visualized with an anisaldehyde-H_2SO_4 spray reagent. IR spectra (wavenumbers in cm^{-1}) were recorded using a Shimadzu IR Prestige-21. Optical rotation measurements were carried out on WXG-4 polarimeter**.** ^{1}H NMR spectra were recorded in deuteriochloroform with tetramethylsilane as an internal standard reference at 300 MHz with a Varian Mercury 300 spectrometer. ^{13}C NMR spectra were recorded in deuteriochloroform at 75 MHz with a Varian Mercury 300 spectrometer. Chemical shifts are given in ppm (δ-scale), coupling constants (J) are given in Hz. The fungus *A. tamarii* Kita MRC 72400 was obtained from TUBITAK Marmara Research Center, Food Science and Technology Research Institute, Culture Collection Unit. Gerhardt THO 500 Thermoshake incubator shaker is used for incubations. Biotransformation experiments were monitored by two sets of three different control flasks. The first controls contained liquid medium and substrate. The second controls contained liquid medium and microorganism and the third controls contained only liquid medium. After 7 days of incubation, all controls were harvested and analyzed by TLC.

2.1 Biotransformation of (-)-Myrtenol (1) by *Aspergillus tamarii*

Glucose (50 g), $NaNO_3$ (2 g), KH_2PO_4 (1 g), KCl (0.5 g), $MgSO_4.7H_2O$ (0.5 g) and $FeSO_4.7H_2O$ (0.01 g) were mixed in distilled water (1 L) to prepare the liquid medium for *A. tamarii* MRC 72400. The medium was evenly distributed among 10 culture flask of 250 mL capacity (100 mL in each) and autoclaved for 15 minutes at 121 °C. Spores were transferred aseptically in a laminar flow hood into one of the flasks containing sterile medium and was incubated at 30 °C and 120 rpm for 3 days. Aliquots (1 mL) from this seed flask were transferred aseptically to the remaining culture flasks and grown for 3 days as above. A clear solution in ethanol (10 mL) of the substrate (500 mg, 3.285 mmol) was then distributed among the culture flasks and fermented for further 7 days. The mycelium was filtered and rinsed with ethyl acetate. The broth was then extracted with 1 L of ethyl acetate three times. The organic layer was washed with brine and dried over anhydrous sodium sulfate and concentrated *in vacuo* to afford a brown gum (650 mg) which was chromatographed on silica gel. Elution with 30% ethyl acetate in hexane gave a colorless oily metabolite (80 mg) identified as the remaining starting material by comparison its ^{1}H and ^{13}C NMR spectra with those of an authentic material. Elution with 50% ethyl acetate in hexane gave another colorless oily metabolite (56 mg, 10%) identified as (-)-*p*-menth-1-en-7,8-diol (**3**). $[\alpha]_D^{20}$: -30.0° (*c* 0.1, $CHCl_3$), (lit. [9], $[\alpha]_D^{20}$: -22.4°, $CHCl_3$, c 2.0). IR: 3240, 1665 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 1.17 (3H, s, H-9), 1.18 (3H, s, H-10), 1.54 (1H, m, H-4), 4.00 (2H, bs, H-7), 5.67 (1H, bs, H-2). ^{13}C NMR (75 MHz, $CDCl_3$): (Table).

2.2 Biotransformation of (-)-Nopol (2) by *Aspergillus tamarii*

The biotransformation of (-)-nopol (**2**) (500 mg, 3.0075 mmol) *by A. tamarii* for 7 days was performed as described above and afforded a brown gum (700 mg) which was chromatographed on silica gel. Elution with 30% ethyl acetate in hexane gave a colorless oil (50 mg) identified as the unchanged starting material by comparison its ^{1}H and ^{13}C NMR spectra with those of an authentic material. Elution with 50% ethyl acetate in hexane gave another colorless oil (82 mg, %14,8) identified as (-)-7-hydroxymethyl-1-*p*-menthen-8-ol (**4**). $[\alpha]_D^{20}$: -28.0°(*c* 0.1, $CHCl_3$). IR: 3412, 1665, 1556 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 1.18 (3H, s, H-10), 1.19 (3H, s, H-11), 1.54 (1H, m, H-4), 3.65 (2H, t, J = 6.5 Hz, CH_2OH), 5.52 (1H, bs, H-2). ^{13}C NMR (75 MHz, $CDCl_3$): (Table).

3. RESULTS AND DISCUSSION

The biotransformations of (-)-myrtenol (**1**) and (-)-nopol (**2**) by *Aspergillus tamarii* MRC 72400 afforded cyclobutyl ring opened metabolites. The incubation of (-)-myrtenol **(1)** with *A. tamarii* MRC 72400 for seven days gave the metabolite **3** (Figure **1**). In the ^{1}H NMR spectrum of **3**, there were two closely located methyl groups at δ_H 1.17 (s, 3H, H-9) and 1.18 (s, 3H, H-10). In the ^{13}C NMR spectrum of **3**, there were two methyl group resonances at δ_C 27.23 and 26.19 and a new quaternary carbon resonance at δ_C 73.07, indicating the presence of a four-membered ring opened metabolite with a tertiary hydroxyl group. The ^{13}C NMR spectrum of **3** exhibited resonances for 10 carbon atoms and its DEPT spectra revealed the presence of 2 methyl, 4 methylene and 2 methine carbon atoms. The metabolite **3**

showed a rotation of -30.0°(c 0.1, $CHCl_3$). All these results suggested that this metabolite was (-)-*p*-menth-1-en-7,8-diol (**3**). Its spectra were comparable with the literature values [9]. The exact stereochemistry of **3** was confirmed by comparison its optical rotation with that in the literature [9].

Table ^{13}C NMR data determined in $CDCl_3$ at 75 MHz for compounds **1-4**

Position	Compounds			
	1	**2**	**3**	**4**
	δ_C	δ_C	δ_C	δ_C
1	43.30	45.47	137.20	133.95
2	147.74	144.60	122.63	123.68
3	117.85	119.13	23.52	23.80
4	31.58	31.64	44.94	44.88
5	40.90	40.56	26.50	26.84
6	37.90	37.70	26.50	28.88
7	31.10	31.30	67.06	40.42
8	21.08	21.10	73.07	72.85
9	26.10	26.14	27.23	27.35
10	65.95	40.10	26.19	26.21
11		59.89		
7-CH_2OH				60.13

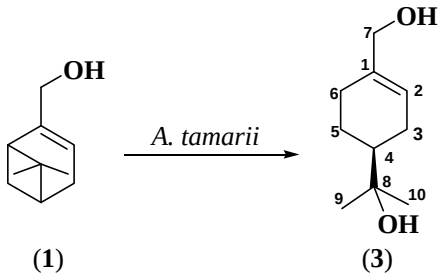

Figure 1. The incubation of (-)-myrtenol (**1**) with *A. tamarii* for 7 days

The biotransformation of (-)-nopol (**2**) by *A. tamarii* MRC 72400 for seven days afforded only the metabolite **4** (Figure **2**). The ^{1}H NMR spectrum of **4** had two methyl resonances at δ_H 1.18 (s, 3H, H-10) and 1.19 (s, 3H, H-11), which were close to each other. The ^{13}C-NMR spectrum of **4** had two methyl resonances at δ_C 27.35 and 26.21 and a new quaternary carbon resonance at δ_C 72.85, showing the presence of a four-membered ring opened metabolite with a tertiary hydroxyl group. The ^{13}C NMR spectrum of **4** displayed resonances for 11 carbon atoms. The presence of 2 methyl, 5 methylene and 2 methine carbon atoms were deduced by its DEPT spectra. The metabolite **4** showed a rotation of -28.0° (c 0.1, $CHCl_3$). All these results suggested that this metabolite was (-)-7-hydroxymethyl-1-*p*-menthen-8-ol (**4**). Its spectra were comparable with the literature values [10].

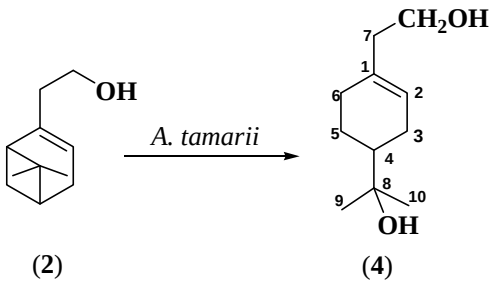

Figure 2. The incubation of (-)-nopol (**2**) with *A. tamarii* for 7 days

In conclusion, the incubations of both (-)-myrtenol (**1**) and (-)-nopol (**2**) by *A. tamarii* MRC 72400 afforded cyclobutyl ring-opened diols **3** and **4**, respectively as in the incubations of the same substrates with *Aspergillus niger* [6].

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