Nat. Volatiles & Essent. Oils, 2016; 3(4): 29-32

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Microbial transformation of 6,6-dimethylbicyclo[3.1.0]hex-2-en-2-ylethanone

Renata Kuriata-Adamusiak[1], Antoni Szumny[2], Gökalp İşcan[3], Fatih Demirci[3*], Stanisław Lochyński[1,4*]

[1] Department of Bioorganic Chemistry, Wrocław University of Technology, Wyb. Wyspiańskiego 27, 50-370 Wrocław, POLAND

[2] Department of Chemistry, Wrocław University of Environmental and Life Sciences, Norwida 25, 50-375 Wrocław, POLAND

[3] Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskisehir, TURKEY

[4] Institute of Cosmetology, Wrocław College of Physiotherapy, Kosciuszki 4, 50-038 Wrocław, POLAND.

*Corresponding author. Email: stanislaw.lochynski@pwr.wroc.pl

Abstract

In this present study, the microbial biotransformation of the volatile carene derivative 6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl-ethanone ($C_{10}H_{14}$) was investigated using 8 different yeast and fungi. Chromatographic and spectroscopic analyses suggested 4 new metabolites.

Keywords: (+)-3-carene, fungal biotransformation, GC/MS

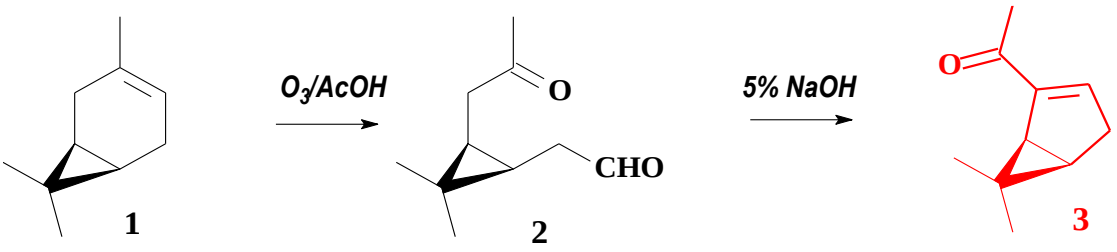
Introduction

The bicyclic monoterpene (+)-3-carene {3,7,7-trimethylbicyclo[4.1.0]hept-3-ene} (**1**), which naturally occurs as a component of allspice (*Pimenta dioica* (L.) Merr.), of one of the major constituent of resinous extract from Scotch pine (*Pinus sylvestris* L.) is used due to its sweet pungent odour as a flavouring agent (Yannai, 2004).

Microbial transformation is an effective tool for the structural modification of bioactive natural and synthetic compounds. Its application in asymmetric synthesis is increasing due to its versatility and ease. Biotransformation of terpenes and terpenoids is a popular route for derivatisation (De Carvalho and da Fonseca, 2006; Paduch et al., 2016; Jivishov et al., 2016).

In our previous reports we presented (Kuriata et al., 2008; Kuriata-Adamusiak et al., 2011; Strub et al., 2014) the bioconversion to the acetate, propionate and butyrate from the mixture of diasteroisomers secondary bicyclo[3.1.0]hexane alcohol (**4**) obtained in three step synthesis from the monoterpene bicyclic hydrocarbon (+)-3-carene (**1**) as seen in Scheme 1.

Scheme 1. The conversion of 3-carene (**1**) to the 6,6-dimethylbicyclo[3.1.0]hex-2-en-2-ylethanone (**3**)



Continuing our studies on the biotransformation of terpenes, the microbial transformation of 6,6-dimethylbicyclo[3.1.0]- hex-2-en-2-ylethanone (**3**) was carried out by 8 microorganisms, to the best of our knowledge for the first time.

Materials and Methods

All microorganisms were cultivated rotatory (200 rpm) at 28 °C for 24-48 hours in 250 mL Erlenmeyer flasks. After the full growth of microorganisms the ethanone (**3**) was added (50 µL) after inclusion to β–cyclodextrin and incubated for 12 days under the same conditions. The biotransformation progress and products were screened by liquid-liquid extraction of the withdrawn broth, which was extracted exhaustively by ethyl acetate, concentrated and evaluated by TLC and GC/MS analyses, respectively. Substrate controls were composed of sterile medium, which the substrate (50 µL) was added and incubated without microorganisms. Culture controls consisted of fermentation blanks, in which the microorganisms were grown under identical conditions but without the addition of substrate. After the incubation period, controls were also harvested and analyzed by TLC. Routine analyses were performed on pre-coated silica gel G-25 UV_{254} plates using *n*-hexane: EtOAc (2:1) as solvent system. Visualization was under UV (254/366 nm) and/or by spraying with anisaldehyde/H_2SO_4 spray reagent (Kuriata-Adamusiak et al, 2011; Jivishov et al., 2016).

Gas chromatography-mass spectrometry analysis conditions

The biotransformation samples were analysed by GC/MS using a Hewlett-Packard GC/MSD system. An Innowax FSC column (60 m x 0.25 mm Ø with 0.25 µm film thickness) was used with Helium as a carrier gas. GC oven temperature was kept at 60 ˚C for 10 min and programmed to 220˚C at a rate of 4˚C/min and then kept constant at 220˚C for 10 min and to 240 ˚C at a rate of 1˚C/min. Split ratio was adjusted at 50:1 with 1 mL/min flow rate. The injector temperature was 250˚C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 425. Relative percentage amounts of the separated compounds were calculated from Total Ion Chromatograms by the computerized integrator.

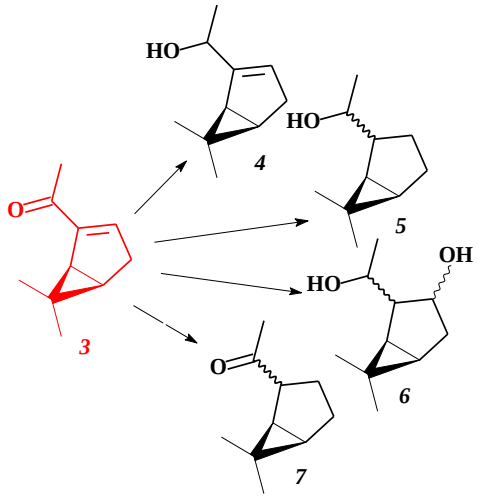
Identification of the metabolites were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) (McLafferty & Stauffer, 1989; König, Joulain, & Hochmuth, 2004) and in-house “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data (Joulain & König, 1998; ESO 2000, 1999), was used for the identification.

Results and Discussion

The key-compound, bicyclic enone (**3**) was synthesized in two step procedure. The ozonolysis of compound **1** followed by intramolecular aldol condensation ketoaldehyde (**2**) afforded desired ketone (**3**), which was distilled under reduced pressure and then purified by means of column chromatography on silica gel (Walkowicz et al., 1981). 6,6-Dimethylbicyclo[3.1.0]hex-2-en-2-ylethanone (**3**) was then subjected to the biotransformation using microorganisms such as *Aspergillus parasiticus, Botrytis cinerea, Absidia blakesleeana, Saccharomyces cerevisiae, Aspergillus niger, Penicillium expansum, Penicillium odoratum,* and *Penicillium adametzii*.

The following metabolites were identified both by GC and GC/MS; 1-(6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)ethanol (**4)**, 1-(6,6-dimethylbicyclo[3.1.0]hexan-2-yl)ethanol **5**, 1-(6,6-dimethylbicyclo[3.1.0]hexan-3-ol-2-yl)ethanol (**6)**, 6,6-dimethylbicyclo[3.1.0]hexan-2-ylethanone (**7**).

Scheme 2. The biotransformation of the enon (**3**) by fungi



	Microorganisms	**Metabolites**
a	*Aspergillus parasiticus*	**4, 5, 6**
b	*Botrytis cinerea*	**4, 5, 7**
c	*Absidia blakesleeana*	**5, 6, 7**
d	*Saccharomyces cerevisiae*	**4, 7**
e	*Aspergillus niger*	**5, 7**
f	*Penicillium expansum*	**5, 7**
g	*Penicillium odoratum*	**7**
h	*Penicillium adametzii*	**7**

Study concerning determination the newly obtained products in stereo aspects and biological activities are still in progress.

ACKNOWLEDGEMENT

This work was presented at the ISEO-2008. Many thanks to Prof. Y. Noma and to Prof. Dr. A.D. Azaz for supplying some of the fungal strains used in the biotransformation experiments.

REFERENCES

De Carvalho, C. C., da Fonseca, M. M. R. (2006). Biotransformation of terpenes. Biotechnology Advances, 24(2), 134-142.

ESO 2000 (1999). The Complete Database of Essential Oils, Boelens Aroma Chemical Information Service, The Netherlands.

Jivishov, E., Kırımer, N., İşcan, G., Demirci, F. (2016). Microbial transformation of p-cymene. Natural Volatiles & Essential Oils, 3(1).

Joulain D, Koenig WA. (1998). The Atlas of Spectral Data of Sesquiterpene Hydrocarbons, EB-Verlag, Hamburg.

Koenig, W.A, Joulain, D., Hochmuth, D.H. (2004). Terpenoids and Related Constituents of Essential Oils. MassFinder 3, Hamburg, Germany.

Kuriata, R., Szumny, A., Iscan, G., Demirci, F., Lochynski, S. (2008). Microbial transformation of bicyclic oxo-derivative obtained from (+)-3-carene, p234, 39th International Symposium on Essential Oils (ISEO 2008), 7-10 September 2008, Quedlinburg, Germany

Kuriata-Adamusiak, R., Strub, D., Szatkowski, P., Lochyński, S. (2011). Biotransformation of bicyclic terpenoid ketones towards new compounds with olfactory properties. Flavour Fragr. J. 26, 351.

McLafferty, F.W; Stauffer, D.B (1989). The Wiley/NBS Registry of Mass Spectral Data, J Wiley and Sons: New York.

Paduch, R., Trytek, M., Król, S. K., Kud, J., Frant, M., Kandefer-Szerszeń, M., & Fiedurek, J. (2016). Biological activity of terpene compounds produced by biotechnological methods. Pharmaceutical Biology, 54(6), 1096-1107.

Strub, D., Balcerzak, L., Lochyński, S. (2014). (+)-3-Carene: valuable starting material for synthesis of low-molecular compounds with olfactory properties. Curr. Org. Chem. 18, 446

Walkowicz M., Walkowicz C, Lochyński S., (1981). Synteza mieszaniny diastereoizomerycznych alkoholi allilowych *E,Z* z układem *gem*-dimetylobicyklo[3.1.0]heksanu, Polish J. Chem. 55, 2007-2013.

Yannai, S. (2004) Dictionary of food compounds with CD-ROM: Additives, flavors, and ingredients. Boca Raton: Chapman & Hall/CRC.