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Microbial transformation of sesquiterpenoid ketone, (+) Nootkatone by *Macrophomia phaseolina*

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Abstrac.: Microbial transformation is an effective tool for the structural modification of bioactive natural and synthetic compounds leading to synthesis of more potent derivatives. Its application in asymmetric synthesis is increasing due to its versatility and ease. This article presents biotransformation of sesquiterpenoid ketone, (+)-Nootkatone (**1**) by *M. phaseolina*, a plant pathogenic fungus. The transformation afforded four main compounds. They were determined to be 1:6 stereoisomeric mixture of 11,12-dihydroxy- 11,12-dihydronootkatone (**2**, **3**), 13-hydroxynootkaone (**4**) and 12-hydroxy-11,12-dihydronootkatone (**5**) with the help of EI-MS, HR-FAB-MS(pos), HR-FAB-MS (neg), 1H-NMR, 13CNMR, COSY-450, NOESY, HMBC, HMQC spectral analyses. The compound **4** was first-chandana-amarasingha-samayawardana-avifauna-Bundala-1.1-28.07 identified as Nootkatone metabolites in this study. Further, the parental compound (**1**) and the transformed products **4** and **5** were found to be present significant antiprotozoal activity.

Key words : Microbial transformation, Nootkatone, *M. phaseolina*

1. Introduction

Microbial transformation of secondary metabolites such as terpenoids, steroids and aromatic compounds from crude drugs and liverworts to obtain potent biologically active compounds such as drugs, pheromones and aromatics have been paid much interest since recently (Choudhary *et al*., 2004: Furusawa *et al*., 2005). Microbial transformation involves the use of different microbes to perform chemical reactions in which the starting substances and product are of comparable chemical complexity. Enzymes in microbes are capable of performing a diverse range of reactions including the insertion of oxygen into C-H and C-C bonds, the addition of oxygen to alkenes, transfer of acetyl or sugar units from one substrate to another, the hydrolysis or formation of amides, epoxides, esters and nitriles. Hydrogenation, hydrolysis and elimination of small units, epimerization and isomarization reactions and the formation of C-C, C-O, C-S and C-N bonds etc. (Devkota *et al*., 2007). As enzymes are chiral biomolecules, these transformatiVajira-

manuscript-RJS-reopened-Nov-2011ons take place with high stereo and enantioselectivity. Biological properties of the compounds mainly depend Vajira-manuscript-RJS-reopened-Nov-2011on the specific configuration of the one or more chiral centers in such microbial transformations get much attention as support in structural modification of natural and synthetic compounds. Moreover, it is capable of inserting functionalities into the inaccessible site of the molecules in which cannot be reached by chemical syntheses and it is low cost and ease of handling. Terpene hydrocarbons and their oxyfunctionalized derivatives, the terpenoids are the most diverse class of natural compounds whereas terpenoids are extensively applied in industry as fragrances and flavours, moreover those are important as chiral synthones for chemical synthesis (Shaw, 1981). Several terpenoids are easily available in large amount from plants or chemical syntheses. Terpene skeleton is most favourable for structural modification by microbes. Numerous publications described already microbial transformation of terpenoids (Bock *et al*., 1988; Bock *et al*., 2006; Ishida, 2005; Joglekar *et al*., 1969; Welf-Rainer-Abraham *et al*., 1986; Hieda *et al*., 1983).

Nootkatone (**1**) is a sesquiterpenoid kVajira-manuscript-RJS-reopened-Nov-2011etone and naturally available in grapefruit essential oil, and it has high demand in cosmetic and fibre industries. It has been reported that expensive aromatics, Nootkatone (**1**) is capable of decreasing somatic fat ratio (Furusawa *et al*., 2005) and posses some repellent activities towards termite (Zhu *et al*.,2001). The cheap aromatic, Velencene from Valencia orange has been successfully converted to Nootkatone (**1**) via biotransformation using different microbes (Furusawa *et al*., 2005: Del Rio *et al*., 1991: Wilson *et al*., 1978). There are few reports on microbial transformation of Nootkatone (**1**). Recentlly (Furusawa *et al*. 2005) have obtained structurally interesting metabolites from Nootkatone by the action of *Aspegillus niger. Fusarium culmorum, Botryosphaeria dothida* (Furusawa *et al*., 2005),

This article deals with identification and testing of biological activities of the metabolites formed by *M.phaseolina*, a plant pathogenchandana-amarasingha-samayawardana-avifauna-Bundala-1.1-28.07 in its transformation of Nootkatone (**1**).

2. Experimental

2.1 Preparation of fermentation medium for biotransformation

M.phaseolina was inoculated and cultivated in Saouraud-Glucose-Agar and stored in refrigerator at 40C for 2 days. The medium for biotransformation was prepared by dissolving glucose (30 g), peptone (15 g), KH_2PO_4 (15 g), yeast extract (15 g), glycerol (30 mL) in 3 L of distilled water. The fermentation medium was distributed among 30 flasks of 250 mL capacity by adding 100 mL in each and autoclaved at 121^0C for 20 min. The spores of *M. phaseolina* were aseptically transferred into the two broth flasks (seed flasks) and cultivated in a shaking table at room temperature for 3 days. *M. phaseolina* in the seed flasks were used for inoculation of the rest of 28 broth flasks and incubated them in the shaking table at room temperature for 3 days.

2.2 Biotransformation of Nootkatone (1)

A commercial sample of (+)-Nootkatone (**1**) (425 mg, Fluka, MF C15H2O, MW 218.338) was dissolved in distilled acetone (15 mL). The solution was evenly distributed among 29 flasks (14 mg / 0.5 mL in each flask) and further incubated for 4 days.

2.3 Isolation of metabolites

After four days of incubation, the culture media and the mycelium were separated by filtration and the filtrate was extracted with dichloromethane three times (1000 mL each case). The combined organic layers was washed with brine and dried over anhydrous $MgSO_4$ and then evaporated under *vaccuo*. The crude extract was purified by column chromatography (*n*-hexane: dicholomethane gradient) to afford pure metabolites Their structures were elucidated with the help of EI-MS, HR-FAB-MS(pos), HR-FABMS (neg), ^{1}H-NMR, ^{13}C-NMR, COSY-45^{0}, NOESY, HMBC, HMQC.

2.4 Testing of biological activities

Commercial sample of (+)-Nootkatone (**1**) and its metabolites **4** and **5** were tested for antibacterial, antiprotozoal, phytotoxic, insecticidal and enzyme inhibition activities.

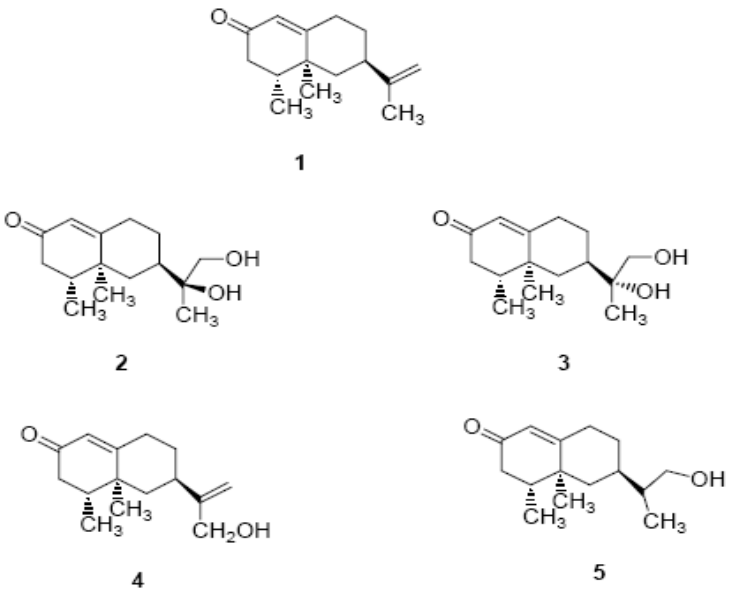

Figure 1: (+)-Nootkatone and its metabolites

3. Results and Discussion

Mainly four compounds were recognized as metabolites (Figure 1); C-11 stereoisomeric mixture of 11, 12-dihydroxy-11, 12-dihydronootkatone (**2, 3**) (yellow oil, 66% isolated yield), 13-hydroxynootkaone (**4**) (pale yellow oil, 12 % isolated yield), 12-hydroxy-11, 12- dihydronootkatone (**5**) (yellow oil, 15% isolated yield) respectively. The compound **4** and **5** were afforded as single stereoisomers. The relative configurations at stereogenic centres of each isomer were determined with the help of 2D NMR data. The stereoi-

somereic ratio of compound **2** and **3** was determined to be 1:6 by using their ^{1}H-NMR spectra.

Table 1: 600MHz 1H-NMR spectral data of compounds 2-5 in CDCl3

Position	2 and 3	4	5
1	5.73 br s	5.75 br s	5.73 br s
3α	2.74 dd	2.27 dd	2.28 dd
3β	2.2 ddd	2.23 ddd	2.22 ddd
4	2.01 m	1.98 m	1.98 m
6 α	2.13 ddd	2.01 ddd	1.90 ddd
6 β	1.06 dd	1.33 dd	0.95 dd
7	1.98 m	2.23 m	1.83 m
8 α	1.72 m	1.98 m	1.86 m
8 β	1.22 m	1.34 m	1.23 m
9 α	2.46 m	2.47 m	2.47 m
9 β	2.27 m	2.45 m	2.35 m
12	3.45 d	5.06 d	3.49 dd
12	3.61 d	4.89 d	3.61 dd
13	1.03 s	4.13 br s	0.93 d
14	0.97 d	0.95 d	0.97 d
15	1.09 s	1.10 s	1.09 s

The spectral data of 1H-NMR and 13C-NMR of each compound are given in Table 1 and Table 2 respectively. The compounds **2**, **3** and **5** have already been reported as Nootkatone metabolites (Mai Furusawa et al., 2005) whereas the compound **4** was first identified as Nootkatone metabolite in this study. (+)-Nootkatone and the metabolite **5** showed to be possessed only antiprotozoal activity in their biological activities testing.

Table 2: 400MHz 13C-NMR data of compound 2-5, in CDCl3

Position	2 and 3	4	5
1	124.38	124.76	124.42
2	199.7	199.63	199.78
3	42.01	42.01	42.03
4	40.54	40.37	40.50
5	39.28	38.58	39.18
6	38.65	45.49	41.26
7	39.20	35.96	34.25
8	27.90	29.67	30.67
9	32.88	33.04	33.10
10	171.00	170.19	171.20
11	74.15	152.53	40.17
12	68.38	109.10	65.82
13	19.77	65.25	13.58
14	14.92	14.93	14.88
15	16.72	16.91	16.77

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Position	2 and 3	4	5
1	5.73 br s	5.75 br s	5.73 br s
3α	2.74 dd	2.27 dd	2.28 dd
3β	2.2 ddd	2.23 ddd	2.22 ddd
4	2.01 m	1.98 m	1.98 m
6 α	2.13 ddd	2.01 ddd	1.90 ddd
6 β	1.06 dd	1.33 dd	0.95 dd
7	1.98 m	2.23 m	1.83 m
8 α	1.72 m	1.98 m	1.86 m
8 β	1.22 m	1.34 m	1.23 m
9 α	2.46 m	2.47 m	2.47 m
9 β	2.27 m	2.45 m	2.35 m
12	3.45 d	5.06 d	3.49 dd
12	3.61 d	4.89 d	3.61 dd
13	1.03 s	4.13 br s	0.93 d
14	0.97 d	0.95 d	0.97 d
15	1.09 s	1.10 s	1.09 s

The possible reactions types in this transformation are shown in Figure 2. In general, oxidation reactions and hydroxylation reactions are more common in terpenoid nuclei during the enzymatic transformations.

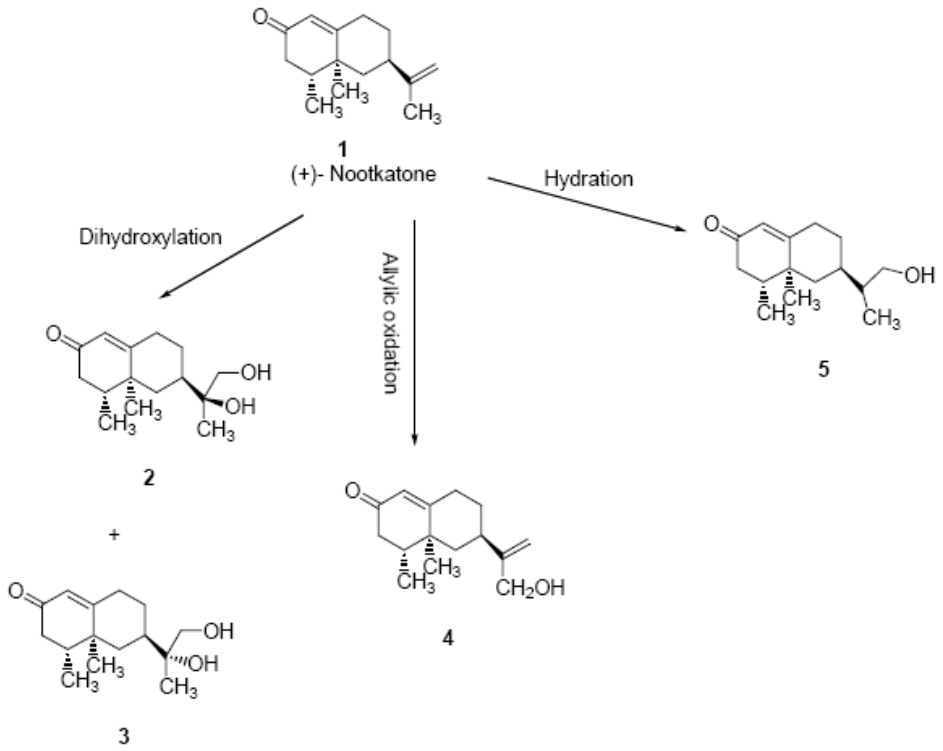

Figure 2 : possible reaction types in biotransformation of Nootkatone by *M. phaseolina*

4. Conclusion

Microbial transformation is a good tool for the structural modification of natural and synthetic compounds with higher stereoselectivity, leading to synthesis of potent derivatives of biological important compounds. This is an ease of handling, low cost, environmentally friendly process. The structure and the stereochemistry of the metabolites depend on the fungal species used in biotransformation. The action of *M.phaseolina* on Nootkatone (**1**) produced four main compounds; the compounds **2**, and **3** as an inseparable 1:6 mixture of stereoisomers and the compounds **4** and **5** as single stereoisomers. Out of these four compounds, the compounds **4** was first identified as a 9 Nootkatone metabolite in our study. The parental compounds (**1)** and the compounds **4** and **5** showed antiprotozoan activity.

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