Biotransformation of germacranolide from *Onopordon leptolepies* by *Aspergillus niger*

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Abstract: Terpenes are present in the essential oils obtained from herbs and spices. They are produced by these plant species as a chemical defense mechanism against phytopathogenic microorganisms. Therefore, terpenes have attracted great attention in the food industry, e.g., they have been used in foods such as cheese as natural preservatives to prevent fungal growth. Herein, we describe the microbial transformation of onopordopicrin (**1**) by *Aspergillus niger*. Four product 11α H-dihydroonopordopicrin (**2**), 11β H–dihydroonopordopicrin (**3**), 3β–hydroxy-11β H-dihydroonopordopicrin (**4**), and 14-hydroxy-11β H-dihydroonopordopicrin (**5**) were obtained. Their structures were identified on the basis of chemical and spectroscopic data. All the four compounds were novel.

Keywords: Onopordopicrin; Hydroxylation; *Onopordon leptolepis*; Asteraceae; Microbial transformation.

INTRODUCTION

Microorganisms can transform a huge variety of organic compounds such as terpene hydrocarbons, alkaloids, steroids, antibiotics, and amino acids (Kieslich, 1976). Onopordopicrin (**1**), a germacranolide sesquiterpene lactone, is a major constituent of *Onopordon leptolepis* DC (Rustaiyan *et al*., 1979), *Onopordon carmanicum* (Bornm.) Bornm. (Rustaiyan and Ganji, 1988), and several other species of the genus *Onopordon* [family Compositae (Asteraceae) (Drozdz, *et al*., 1958; Drozdz and Piotrowski, 1973). Sesquiterpene lactones have been reported to possess several biological properties including cytotoxic, antibacterial, anti-inflammatory, antimalarial, hypotensive and etc. (Heilmann, 2001; Qinghaosu, 1977; Qinghaosu, 1979). Germacranolides represent one of the largest groups of sesquiterpene lactones, including over 1000 known naturally occurring compounds. Much attention has been focused on their antitumor properties. Germacranolides are common constituents of most Iranian genera of the compositae (Rustaiyan *et al*., 1990; Rustaiyan *et al*., 1991; Rustaiyan and Ganji, 1988).

Herein, we describe the biotransformation of onopordopicrin (a germacranolide sesquiterpene lactone). In addition, the fermentation methods, isolation, and structure elucidation of the metabolite are also reported. To the best of our knowledge, this is the first report regarding the microbial metabolism of onopordopicrin.

MATERIALS AND METHODS

General

Onopordopicrin (**1**) was isolated and purified from the aerial parts of *Onopordon leptolepis* (Rustaiyan *et al*., 1979). IR spectra were recorded in KBr using a PYE infrared spectrometer, and specific rotations were obtained at the ambient temperature on a Perkin-Elmer polarimeter model 241 MC.

The ^{1}H-^{13}C-NMR spectra were obtained in $CDCl_3$ on a Bruker DRX-500 NMR spectrometer operating at 500 and 125 MHz, respectively. The chemical shift values are reported as ppm (δ) using tetramethylsilane (TMS) as the internal standard, and coupling constants are expressed in Hz. Mass spectra were recorded on a Shimadzu QP500 OGC/mass spectrometer. Thin layer chromatographic analyses were carried out on pre-coated silica gel 60 F_{254} (Merck) using n-hexane-EtOAc mixtures as solvent systems, and spots were visualized under short-wavelength UV light or by spraying $KMnO_4$ or vanillin–H_2SO_4 spray reagent. The adsorbent used for column chromatography was silica gel 60/230-400 mesh (Merk).

Microorganisms and cultural condition

A strain of *Aspergillus niger* was isolated in our laboratories from soil in Tehran prefecture and was identified by its physiological and morphological characteristics according to the Persian Type Culture Collection (P.T.C.C.5011), at the Iranian Research Organization for Science and Technology, Tehran, Iran. A Czapek-peptone medium [1.5% sucrose, 1.5% glucose, 0.5% polypeptone, 0.1% K_2HPO_4, 0.05% KCl, and 0.001% $FeSO_4.7H_2O$ in distilled water (pH 7.0)] was used for the biotransformation of the substrate by the microorganism.

Biotransformation of Onopordopicrin by A. niger

Aspergillus niger was inoculated and cultivated rotatory (100 rpm) in Czapek-peptone medium (pH 7.0 at 30°C) for 2 days. Onopordopicrin (**1**) (100 mg/200mL) was added to the medium and further cultivated for 3 days

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After the completion of the incubation time, the culture was filtered *in vacuum* and the broth was extracted with EtOAc (3 ×100 mL). The extracts were dried over $MgSO_4$ and the solvent was evaporated *in vacuum* to give the crude extract. The sample extraction were chromatographed with silica gel column by *n*-hexane-methanol-EtOAc gradient to give 14 fractions. These were grouped according to their TLC profiles. Fractions 8–13 (70 mg) were reunited and rechromatographed on the Slica gel (230-400 mesh) to give 11α H-dihydroonopordopicrin (**2**) (6.6 mg) and 11β H-dihydroonopordopicrin (**3**) (4.6 mg). Fractions 11–13 (11.2 mg) were combined and portion of 3β-hydroxy-11β-H-dihydroonopordopicrin (**4**) (7.6 mg) was processed by HPLC using a C_{18} column ($MeOH/H_2O$ 6:4) at a flow rate of 3 ml/min to give 100 mg of (**4**). 11β H–dihydroonopordopicrin (**3**) extracted by column chromatography on the Slica gel and preparative HPLC to afford (1.5 mg) (**5**).

Compound **1**. Oil; m/z (% relative intensity) (HR-EIMS; $[M^+]$ m/z 333.0128): $[\alpha]_\alpha$ $+27.7^0$ (0.062; MeOH); IR ν_{max} cm^{-1}: 3400, 1715, 1640; ^{1}H NMR (500 MHz, $CDCl_3$ TMS): δ 1.50 (1H, s, H-14), 1.90 (1H, m, H-9β), 2.10 (1H, m, H-9α), 2.20 (1H, m, H-2), 2.50 (1H, m, H-3′), 2.60 (1H, m, H-2′), 2.60 (1H, m, H-3), 3.00 (1H, m, H-7), 4.00 (1H, d, 11.7, H-15′), 4.10 (1H, d, 9.0, H-6), 4.30 (1H, d, 11.8, H-15), 5.02 (1H, ddbr, 4.0, 12.2, H-1), 5.10 (1-H, ddbr, 11.0, 11.1, H-8), 5.13 (1H, d, 8.9, H-5), 5.70 (1H, d, 3.1, H-13′), 5.91 (1H, sbr, H-4′), 6.20 (1H, d, 3.6, H-13), 6.35 (sbr, H-4); ^{13}C NMR: (125 MHz, $CDCl_3$ TMS): δ 17.1 (q, C-14), 21.1 (t, C-2), 26.5 (t, C-3), 49.0 (m, C-9), 53.3 (d, C-7), 61.3 (t, C-3′), 62.2 (t, C-15), 63.9 (d, C-6), 73.4 (d, C-8), 125.9 (s, C-11) 126.9 (d, C-1), 128.7 (t, C-4′), 130.1 (d, C-5), 132.6 (s, C-10), 135.7 (s, C-4), 139.7 (s, C-2′), 144.5 (q, C-13). 165.4 (s, C-12), 170.5 (s, C-1′).

Compound **2**. Oil; m/z (% relative intensity) (HR-EIMS; $[M^+]$ m/z 333.0018): $[\alpha]_\alpha$ +27.7° (0.066; MeOH); IR ν_{max} cm^{-1}: 3120, 1727, 1657; ^{1}H NMR (500 MHz, $CDCl_3$ TMS): δ 1.30 (1H, d, 3.5, H-13), 1.53 (1H, sbr, H-14), 2.20 (1H, m, H-2), 2.21 (1H, m, H-2′), 2.21 (1H, m, H-3), 2.50 (1H, m, H-3′), 2.50 (1H, dd, 11.0, 12.5, H-9α), 2.70 (1H, dd, H-9β), 1.90 (1H, m, H-11), 4.20 (1H, d, 10.9, H-15′), 4.30 (1H, d, 11.7, H-15), 5.90 (1H, sbr, H-4′), 4.70 (1H, dd, 8.9, 8.3, H-6), 3.10 (1H, m, H-7), 5.08 (1H, dd, 2.3, 11.9, H-1), 5.20 (1H, d, 9.0, H-5), 5.21 (1H, ddd, 11.5, 11.5, 5.0, H-8), 6.30 (1H, sbr, H-4). ^{13}C NMR (125 MHz, $CDCl_3$ TMS): δ 16.9 (q, C-14), 17.1 (q, C-13), 26.3 (t, C-2), 34.9 (t, C-3), 40.4 (d, C-11), 49.3 (m, C-9), 58.8 (d, C-7), 61.0 (t, C-3′), 61.9 (t, C-15), 73.8 (d, C-8), 76.8 (d, C-6), 124.3 (t, C-4′), 126.5 (d, C-1), 129.6 (d, C-5), 132.9 (s, C-10), 139.9 (s, C-4), 143.5 (s, C-2′), 165.6 (s, C-12), 179.2 (s, C-1′).

Compound **3**. Oil; m/z (% relative intensity) (HR-EIMS; $[M^+]$ m/z 333.1128): $[\alpha]_\alpha$ +27.7° (0.062; MeOH); IR ν_{max} cm^{-1}: 3420, 1767, 1627; ^{1}H NMR (500 MHz, $CDCl_3$ TMS): δ 1.10 (1H, d, 3.7, H-13), 1.60 (1H, sbr, H-14), 1.85 (1H, m, H-11), 2.0 (1H, m, H-9α), 2.20 (1H, m, H-9β), 2.20 (1H, m, H-3), 2.30 (1H, m, H-2′), 2.40 (1H, m, H-2), 2.40 (1H, m, H-3′), 3.09 (1H, m, H-7), 4.20 (1H, d, 10.8, H-15′), 4.25 (1H, d, 12.1, H-15), 4.50 (1H, dd, 8.3, 9.0, H-6), 5.08 (1H, dbr, 10.1, H-1), 5.10 (1H, m, H-5), 5.40 (1H, ddbr, 10.5, 11.0, H-8), 5.91 (1H, sbr, H-4′), 6.25 (1H, sbr, H-4). ^{13}C NMR (125 MHz, $CDCl_3$ TMS): δ 17.0 (q, C-14), 17.3 (q, C-13), 26.4 (t, C-2), 35.1(t, C-3), 41.4 (d, C-11), 49.3 (m, C-9), 58.6 (d, C-7), 61.5 (t, C-3′), 62.5 (t, C-15), 74.1 (d, C-8), 76.7 (d, C-6), 124.5 (t, C-4′), 126.8 (d, C-1), 129.9 (d, C-5), 133.9 (s, C-10), 139.7 (s, C-4), 143.4 (s, C-2′), 165.7 (s, C-12), 178.9 (s, C-1′).

Compound **4**. EIMS: Oil; m/z (% relative intensity) (HR-EIMS; $[M^+]$ m/z 350.4007): $[\alpha]_\alpha$ +27.7° (0.090; MeOH); IR ν_{max} cm^{-1}: 3350, 1718, 1667; ^{1}H NMR (500 MHz, $CDCl_3$ TMS): δ 1.10 (1H, d, 3.7, H-13), 1.60 (1H, sbr, H-14), 1.85 (1H, m, H-11), 2.0 (1H, m, H-2), 2.0 (1H, m, H-2′), 2.0 (1H, m, H-9α), 2.20 (1H, m, H-9β), 3.09 (1H, m, H-7), 4.20 (1H, dd, 9.5, 6.9, H-3), 4.20 (1H, d, 10.8, H-15′), 5.10 (1H, d, 8.9, H-5), 4.50 (1H, dd, 8.3, 9.0, H-6), 4.25 (1H, d, 12.1, H-15), 5.11 (1H, ddbr, 1.23, 1.2, H-1), 5.40 (1H, ddbr, 10.5, 11.0, H-8), 5.91 (1H, sbr, H-4′), 6.25 (1H, sbr, H-4). ^{13}C NMR (125 MHz, $CDCl_3$ TMS): δ 17.0 (q, C-14), 17.3 (q, C-13), 26.4 (t, C-2), 49.3 (m, C-9), 58.6 (d, C-7), 61.5 (t, C-3′), 62.5 (t, C-15), 69.9 (d, C-3), 74.1 (d, C-8), 76.7 (d, C-6), 124.5 (t, C-4′), 126.8 (d, C-1), 129.9 (d, C-5), 133.9 (s, C-10), 139.7 (s, C-4), 143.4 (s, C-2′), 165.7 (s, C-12), 178.9 (s, C-1′).

Compound ***5***. Oil; EIMS: m/z (% relative intensity) (HR-EIMS; $[M^+]$ m/z 350.4108): $[\alpha]_\alpha$ +27.7° (0.084; MeOH); IR ν_{max} cm^{-1}: 3470, 1768, 1630; ^{1}H NMR (500 MHz, $CDCl_3$ TMS): 1.40 (1H, d, 3.6, H-14), 1.45 (1H, sbr, H-14), 1.70 (1H, m, H-11), 2.00 (1H, m, H-9′), 2.01 (1H, m, H-2), 2.10 (1H, m, H-9), 2.20 (1H, m, H-2′), 4.00 (1H, m, H-7), 4.10 (1H, m, H-3), 4.10 (1H, d, 12.3, H-15), 4.20 (1H, d, 11.3, H-15′), 4.30 (1H, dd, 9.1, 8.1, H-6), 5.09 (1H, ddbr, 3.9, 12.4, H-1), 5.20 (1-H, ddbr, 12.0, 11.9, H-8), 5.22 (1H, d, 9.1, H-5), 5.90 (1H, d, 3.1, H-4′), 5.91 (1H, d, H-4); ^{13}C NMR: (125 MHz, $CDCl_3$ TMS): δ 14.4 (q, C-13), 23.1 (t, C-2), 40.4 (t, C-9), 40.6 (d, C-11), 58.7 (d, C-7), 60.8 (d, C-3), 61.7 (t, C-3’), 62.6 (t, C-15), 64.8 (t, C-14), 71.3 (d, C-8), 76.6 (d, C-6), 129.4 (t, C-4′), 129.9 (s, C-5), 128.8 (d, C-1), 133.0 (s, C-10), 139.7 (d, C-4), 143.4 (s, C-2′), 165.7 (s, C-12), 178.7(s, C-1′).

RESULTS

Onopordopicrin (**1**) was isolated from a $CHCl_3$:CH_3OH (3:1) extract of *Onopordon leptolepis*. Biotransformation of (**1**) by *A. niger* afforded several metabolites (fig. 1). Incubation of **(1)** with *A. niger* for 3 days at 30°C in a

rotary shaker operating at 100 rpm led to the formation of compounds (**2**), (**3**), and (**4**). Metabolites (**2**) and (**3**) had the same molecular formula, $C_{19}H_{26}O_6$, as deduced by HR-EIMS. ^{1}H and ^{13}C-NMR spectra for (**3**) were generally similar to those for (**2**), except for some minor differences. Examination of the ^{1}H-NMR spectrum did not show the two downfield doublets at δ 6.20 and 5.70 ppm corresponding to the exocyclic methylene γ-lactone in (**1**). However, a new methyl signal at δ 1.30 (J = 6.8 Hz) attributed to H-13 was observed. Most notably, the (C-13) ^{13}C-NMR signal in (**3**) resonated at δ 17.10 ppm, versus 17.30 in (**2**). Further, (H-7) in (**2**) was more deshielded than the same proton signal (H-7) in (**3**)**.** It could also be seen that (H-13) was more shielded in (**2**) than in (**3**). These observations strongly suggest that (**2**) was epimeric with (**3**) at (C-11).

Metabolism of onopordopicrin by *A. niger* gave, in addition to (**2**) and (**3**), a more polar germacranolide compound (**4**) in a 0.3% yield. Compound (**4**) was obtained as a colorless oil, whose molecular formula $C_{19}H_{26}O_7$ was established by HR–EIMS (M^+ m/z 350.4007). In the IR spectrum of (**4**), absorptions indicative of hydroxyl, lactone, and ester functionalities were observed at 3450, 1765, and 1730 cm^{-1}, respectively. The spectroscopic data of (**4**) were quite similar to those of (**3**), except for the presence of a methine group bearing an allylic hydroxyl group (δ_H 4.20, 1H, dd, J = 9.5, 6.9 Hz; δ_c 69.9d), suggesting the introduction of a hydroxyl group at (C-3). This was further confirmed by the ^{1}H-^{1}H 2D COSY NMR spectrum of (**4**), in which the correlation between (H-3), (H-2), and between (H-2) and (H-1) was observed. The stereochemistry of the hydroxyl group was suggested to be β-oriented, as the coupling constants of (H-3) were J = 9.5 and 6.9 Hz. This assumption was further confirmed by the NOEs between (H-3)/(H-2α), (H-7)/(H-13), and (H-9α) in the NOESY spectrum (fig. 2). Further incubation of 11β H–dihydro onopordopicrin (**3**) using the organism (*A. niger*) for 3 days at 30°C led to the formation of compound (**5**), a very polar metabolite.

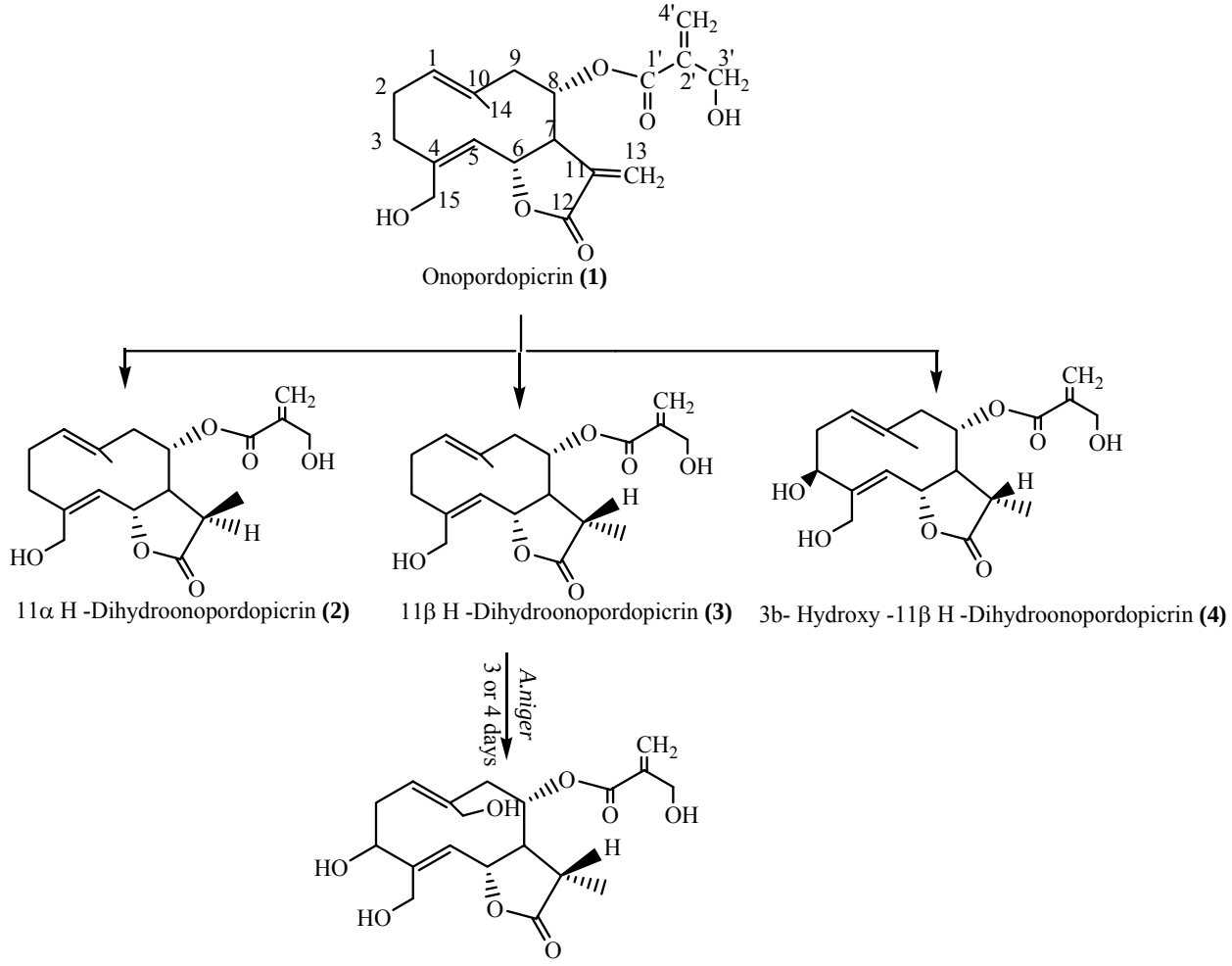

Fig. 1: Biotransformation of Onopordopicrin **(1)** by *Aspergillus niger*

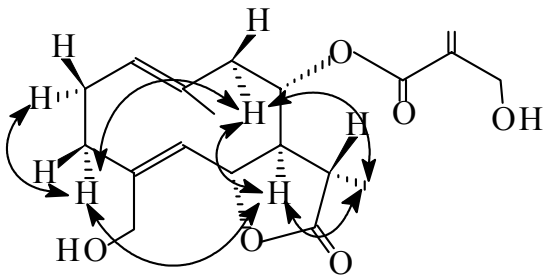

Fig. 2: Selected NOE effects for 3β-Hydroxy-11β H-Dihydroonopordopicrin **(4)** (from NOESY, ↔)

Compound (**5**) had the same molecular formula, $C_{19}H_{26}O_7$ (HR-EIMS; [M^+] m/z 350.4108), as that of compound (**4**). The ^{13}C-NMR spectrum of (**5**) lacked the C-14 methyl signal but instead exhibited a hydroxymethyl group at δ 70.8(t). The ^{1}H-NMR spectrum of (**5**) showed, in addition to the two already existing primary alcohols, a third hydroxyl group δ_H 4.30, 4.20 (2H each d, J = 12.6 Hz).

DISCUSSION

Sesquiterpene lactones are present in the essential oils obtained from herbs and spices. They are produced by these plant species as a chemical defense mechanism against phytopathogenic microorganisms. Previously, studies have been conducted on the biotransformation of citral of *Penicillium* sp. and microbial transformation of citral of *Penicillium digitatum* (Wood, 1969). Microbial transformation of geraniol, nerol, and citral by *A. niger* produced linalool and α-terpineol. Bioconversion of nerol with *Penicillium chrysogenum* yielded mainly α-terpineol and some unidentified compounds. With *Penicillium rugulosum,* the major bioconversion product from nerol and citral was linalool (Wood, 1969). In previous studies of bioconversion of citral and nerol by spores of *P. digitatum,* these were transformed into 6-methylhept-5-en-2-one by sporulated surface cultures (Wood, 1969). Microbial transformation of geraniol, nerol, and citral by *A. niger* produced linalool and α-terpineol. Bioconversion of nerol with *P. chrysogenum* yielded mainly α-terpineol and some unidentified compounds. With *P. rugulosum* the major bioconversion product from nerol and citral was linalool (Wood, 1969). The study of microbial transformation of menthol by sporulated surface cultures of *A. niger* and *Penicillium* sp. produced *cis-p*-menthan-7-ol from *A. niger*; the main products obtained were limonene, *p*-cymene, and γ-terpinene by Esmaeili, *et al*. (2009) (Esmaeili, *et al*., 2009a). The two main products of microbial transformation of citral were similar to those obtained in former work. The main bioconversion products of (-)-menthol by *Mucor ramannianus* using the sporulated surface cultures method were *trans-p*-menthan-8-ol, *trans*-menth-2-en-1-ol, sabinane, *p*-menthane-3,8-diol, isomenthol, and 1,8-cineole reported by Esmaeili, *et al*. (2009b). The main biotransformation products obtained from menthol by surface *Penicillium* sp. were α-pinene (18.0%), *trans-p*-menthan-1-ol (10.6%), *p-menth-1-ene* (5.8%), sabinene (3.9%), 1,8-cineole (6.4%), and limonene (3.2%) reported by Esmaeili *et al*. (2009c). Biotransformation of myrcene by *Pseudomonas aeruginosa* converted to dihydrolinalool and 2,6-dimethyloctane in high percentages (Esmaeili and Hashemi, 2011). T*he main* biotransformation of myrcene by *Pseudomonas putida was* dihydrolinalool *(*Esmaeili, *et al*., 2011). The experimental work (four latest articles) suggested that biotransformation of monoterpenes with *different* fungi caused an oxidation reaction and resulted in a more stable product. But bioconversion onopordopicrin using *A. niger* showed it was possible to obtain products which have not been previously reported. It is describe the microbial transformation of onopordopicrin (**1**) by *A. niger*. Four product 11α H-dihydroonopordopicrin (**2**), 11β H–dihydroonopordopicrin (**3**), 3β–hydroxy-11β H-dihydroonopordopicrin (**4**), and 14-hydroxy-11βH-dihydroonopordopicrin (**5**) were obtained. Biotransformation *A. niger* showed, this fungi caused an oxidation or reduction reaction which resulted in a more stable product. 11α H-dihydroonopordopicrin (**2**) and 11β H–dihydroonopordopicrin produced obtained with higher yield. But 11β H–dihydroonopordopicrin (**3**) and 3β–hydroxy-11βH-dihydroonopordopicrin (**4**) oxidation products have lower yield. The experimental work suggested that biotransformation by *A. niger* oxidation are main products.

REFERENCES

Drozdz B and Piotrowski J (1973). Sesquiterpene Lactones Part 6 Lactones of Carduinae Subtribe. *Polish J. of Pharm. and Pharmacy*, **25**: 91-94.

Esmaeili A, Safaiyan S Sharafian S, and Rustaiyan A (2009a). Biotransformation of one monoterpene by sporulated surface culture of *Aspergillus niger* and *Penicillium* sp. Nat. Prod. Res., **23**(11): 1058-1061.

Esmaeili A, Saad N, Safaiyan S and Rustaiyan A (2009b) Biotransformation of (-)-menthol by *Mucor ramannianu* and Study of the Pathways Involved. *Herba Pol.,* **55**(2): 51-58.

Esmaeili A, Hoseiny Zarea A, Sharafian S, Safaiyan S and Rustaiyan A (2009c). Biotransformation of one monoterpene by sporulated surface cultures of *Aspergillus niger* and *Penicillium* sp. *Herba Pol.,* **55**(1): 78-83.

Esmaeili A and Hashemi E (2011). Biotransformation of myrcene by *Pseudomonas aeruginosa*. *Chem. Central J.,* **5**: 26-34.

Esmaeili A, Hashemi E, Safaiyan SH and Rustaiyan A (2011). Biotransformation of Myrcene by *Pseudomonas putida* PTCC 1694. *Herba Pol.,* **57**(1): 51-58.

Heilmann J, Wasescha MR and Schmidt TJ (2001). The influence of glutathione and cysteine levels on the cytotoxicity of helenanolide type sesquiterpene lactones against KB cells. *Bio. and Med. Chem*., **9**: 2189-2194.

Kieslich K (1976). Microbial transformations of non-steroid cyclic compounds Georg Thieme Verlag, Stuttgart, Germany, pp.1-10.

Obafemi CA, Sulaimon TO, Akinpelu DA and Olugbade TA (2006). Antimicrobial activity of extracts and a germacranolide-type sesquiterpene lactone from *Tithonia diversifolia* leaf extract. *Afri. J. of Bio.,* **5**(12): 1254-1256.

Qinghaosu A (1977). Antimalarial Co-ordinating Group of Research on the structure of Qing Hau Sau. K'O Hsueh *Tung Pao.,* **22**, 142-144. Chem. Abstr (1977). **87:** 98788 .

Qinghaosu A (1979) Antimalarial co-ordinating Research group. *Chin. Med. J.,* **92**: 811-812.

Rustaiyan A, Ahmadi B, Jakupovic J and Bohlmann F (1986). Sesquiterpene lactones and eudesmane derivatives from *Onopordon carmanicum*. *Phytochem.,* **25**: 1659-1662.

Rustaiyan A, Habibi Z, Saberi M and Jakupovic J (1991). A nor-guaianolide and a glaucolide-like eudesmanolide from *Pulicaria undulata*. *Phytochem.* **30**: 2405-2406.

Rustaiyan A, Nazarians L and Bohlmann F (1979). Two new germacranolides from *Onopordon leptolepis. Phytochem.,* **18**: 883-835.

Rustaiyan A and Ganji M (1988). Germacranolides from Jurinea eriobasis. *Phytochem.,* **27**: 2991-2992.

Rustaiyan A, Zare K, Habibi Z and Haschemi M (1990). Germacranolides from tanacetum polycephalum. *Phytochem.,* **29**: 3022-3023.

Wood JB (1969) Microbial fermentation of lower terpenoids. *Process Biochem.,* **2**: 50-52.