Aszonapyrones from the fungus *Neosartorya tatenoi*

Aszonapyrones จากรา *Neosartorya tatenoi*

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ABSTRACT

Air-dried biomass of the fungus *Neosartorya tatenoi* (109.4 g) was extracted with EtOAc to give crude EtOAc extract 12.8 g (13.9%). Chromatographic separation of this crude extract led to the isolation of two meroterpenoids. Their structures were identified on the basis of spectroscopic methods (IR, MS, ^{1}H NMR, ^{13}C NMR, and 2D NMR) as aszonapyrone A (**I**) and aszonapyrone B (**II**). In addition, their cytotoxicity towards two cancer cell lines was also evaluated.

บทคัดย่อ

การสกัดเชื้อรา *Neosartorya tatenoi* แห้ง 109.4 กรัม ด้วยตัวทำละลายเอธิลอะซิเตต ได้ส่วนสกัดหยาบเอธิลอะซิเตต 12.7 กรัม (13.9 %) เมื่อแยกส่วนสกัดหยาบเอธิลอะซิเตตด้วยวิธีทางโครมาโทกราฟีได้สาร meroterpenoids 2 สาร โครงสร้างของสารทั้งสองพิสูจน์ด้วยวิธีทางสเปกโทรสโกปี (IR, MS, ^{1}H NMR, ^{13}C NMR และ 2D NMR) ได้เป็น aszonapyrone A (**I**) และ aszonapyrone B (**II**) นอกจากนี้ยังได้ตรวจสอบความเป็นพิษต่อเซลล์มะเร็ง 2 ชนิดของสารทั้งสองด้วย

Key Words: *Neosartorya tatenoi*, Meroterpenoid, Aszonapyrone

คำสำคัญ: นีโอซาร์โทรยา ทาเทอนอย มีโรเทอร์ปีนนอยด์ แอสโซนาไพโรน

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1. Introduction

There are new human diseases reported each year. Therefore, drugs research is very important to find the new drugs for protection from these diseases. The new drugs have been reported continually including drug discovery from bioactive compounds from plants and microorganism. At the beginning of the 21st century, fungi were involved in the industrial processing of more than 10 of the 20 most profitable products used in medicine. Many species of the genus *Neosartorya*, the sexual states of *Aspergillus* species, family Trichocomaceae (Samson et al., 2007), have been reported for compounds with bioactivities such as antibacterial, antimalarial and cytotoxicity against cancer cells. For example, a pyrroloindole, fischerindoline, isolated from the solid and liquid cultures of the *Neosartorya pseudofischeri,* inhibited the growth of six human and mouse cancer cell lines *in vitro* (Masi et al., 2013). Three compounds, grabramycins A-C which isolated from *Neosartorya glabra*, were reported as antibacterial (Jayasuriya et al., 2009). The aszomapyrone A, isolated from the culture of the soil fungus *Neosartorya fischeri* (KUFC6344), showed strong growth inhibitory against three cancer cell lines MCF-7, NCI-H460, and A375-C5 (Eamvijarm et al., 2013). Therefore, the searching for bioactive compounds from the fungus *Neosartorya* species is challenging.

Objective of this study

Since there was no report on chemical constituents of the fungus *N. tatenoi*, this research focus on isolation and determination of the chemical constituents and their cytotoxicity against two cancer cell lines from this fungus.

2. Methodology

2.1 Fungal material

The fungus *N. tatenoi* were isolated from the soil collected from forest around the Pha Nok Kao Silvicultural Station, Khon Kaen Province Thailand. The fungus was cultured in conical flasks (500 ml, 75 flasks) with potato dextrose broth (PDB) (200 ml/flask) and incubated in a standing condition at room temperature in darkness for 3 weeks. The culture broth was filtered out to give a wet fungal biomass and then air-dried at room temperature.

2.2 General experimental procedures

Melting points were determined using Electrothermal IA9200 digital melting point apparatus. IR spectra were obtained using a Bruker Tensor 27 spectrophotometer. NMR spectra were recorded on a Varian Mercury Plus 400 spectrometer using $CDCl_3$ and CD_3OD as solvents. The internal standards were referenced from the residue of those solvents. Column chromatography was carried out on MERCK silica gel 60 (230–400 mesh). TLC was performed with precoated MERCK silica gel 60 PF254; the spots were visualized under UV light (254 and 366 nm) and further by spraying with anisaldehyde then heating until charred.

2.3 Extraction and Isolation

The air-dried biomass of the fungus *N. tatenoi* (109.4 g) was ground and extracted with EtOAc (400mL x3) at room temperature. Removal of the extract solvent under reduce pressure gave EtOAc extract, 12.7g (13.9%).

The precipitate in EtOAc extract was filtered out and then recrystallized from CH_2Cl_2 to give colorless needles of **I** (118.5 mg). Filtrate was then separated on silica gel flash column

chromatography (FCC), eluted with a gradient system of hexane:EtOAc and EtOAc:MeOH. A 150 mL of eluent was collected for each fraction to give a total of nine fractions, designated as NtE1-NtE9. The pale brown precipitate of fraction NtE7 was filtered out and was recrystallized from hexane to give colorless needles of an additional amount of **I** (322.9 mg). The pale brown precipitate of fraction NtE8 was filtered out and was recrystallized from CH_2Cl_2 to give colorless needles of **II** (84.4 mg).

2.4 Bioassay Experiments

The bioassay experiment was carried out at the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. The isolated compounds were sent to test for cytotoxicity towards three cancer cells lines, small cell lung cancer (NCI-H187) and epidermoid carcinoma in the mouth (KB).

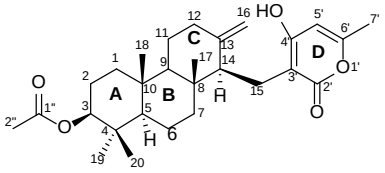
3. Results and Discussion

The crude EtOAc extract obtained from the air-dried biomass of *N. tatenoi* was separated by chromatographic method and two meroterpenoids were isolated. By using of physical and spectroscopic data as well as comparing the data with those of reported in literature, they were identified as aszonapyrone A (**I**) and aszonapyrone B (**II**).

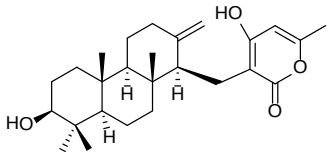
Compound (**I**) was obtained as colorless crystals, mp 241-243 ^{o}C. The ^{13}C NMR and DEPT spectra revealed 28 signals attributable to six methyl, eight methylene, five methine (including alkene) and nine quaternary carbons. The ^{1}H and ^{13}C NMR spectram at δ_H 2.01 (3H, s)/δ_C 21.0 (C-2") and δ_C 171.7 (C-1") together with the IR absorption bands at 1728 cm^{-1} indicated the presence of an acetoxy group. The other four singlet signals of methyl groups of compound **I** (Table 1) showed the NMR signals at δ_H 0.71/δ_C 14.8 (C-17), δ_H 0.76/δ_C 16.1 (C-19), δ_H 0.80/δ_C 16.2 (C-18) and δ_H 0.81/δ_C 27.8 (C-20) together with an oxymethine proton at δ_H 4.42 (t, 8.2, H-3), six methylene protons at δ_H 1.02-2.25, and three methine protons at δ_H 0.91-2.43, providing a most useful indicator for the present of a tricyclic terpenoid (Kanokmedhakul et al., 2011). A terminal methylene group of ring C showed the IR absorption bands at 1642 and 1447 cm^{-1} while the NMR signals showed at δ_H 4.82 and 4.60/δ_C 106.1 (C-16) and δ_C 148.9 (C-13). Two aliphatic methylene protons of C-15 at δ_H 2.57 (1H, m) and 2.47 (1H, m). The ^{1}H NMR signals of α-pyrone, ring D, showed a vinyl proton at δ 5.82 (1H, s, H-5') and a singlet signal a methyl group at δ 2.10 (3H, 7'). The ^{13}C NMR showed five signals characteristic for two oxygenated olefinic carbons at δ 165.7 (C-4') and 159.5 (C-6'), the other olefinic carbons at C-3' and C-5' appeared at δ 102.7 and 100.5, respectively, while a lactone carbonyl exhibited at δ 167.1 (C-2). IR absorption bands also supported the presence of hydroxyl group and carbonyl group at 3480 cm^{-1} and 1671 cm^{-1}, respectively. The COSY exhibited a cross coupling network between H-1/H-2/H-3, H-5/H-6/H-7, H-11/H-12/H-16, H-16/H-14/H-15 and 5'-H/7'-H (allylic coupling). The HMBC spectrum (Fig. 2) exhibited correlation of olefinic proton 5' to C-3', C-4', C-6' and C-7'; and H-7' to C-4' and C-6' to complete the assignment of the α-pyrone ring D and its substituents. The cross-peaks of correlation of H-15 to C-8, C-13, C-14, C-2', C-3', and C-4' extended the connectivity between rings C and D. The correlations of H-7 to C-5, C-8, C-9, C-14, and C-17; and H-14 to C-7, C-13, C-17, and C-16 extended between two fragments of B/C rings junction. In addition, the HMBC spectrum showed correlations of methylene

proton H-1 to C-3, C-5, C-9, and C-18; and H-5 to C-4, C-7, C-18 and C-19, while the correlations of methyl protons H-2" to C-1" and H-3 to C-1" confirmed the position of acetoxyl group in ring A.

The relative stereochemistry was determined by a combination of coupling constant and analysis of the NOESY spectral data (Fig. 3). The 1,3-diaxial NOESY correlation was observed between H-5 and H-3. There was no correlation between H-5 and the methyl group at C-10 (H-18) suggesting the *trans* A/B rings junction. A 1,3-diaxial correlation was observed at H-14 and H-12; H-5 and H-14; and H-5 and H-9 while the correlation between H-14 and H-17 was not observed, indicating the *trans* B/C rings jungtion. From the above spectroscopic analysis, and comparison with those of literature data (Kimura et al., 1982 and Kanokmedhakul el al., 2011), it was found that compound **I** is a meroterpenoid named aszonapyrone A.



aszonapyrone A (**I**)



aszonapyrone B (**II**)

Fig 1 Structures of compounds **I –II.**

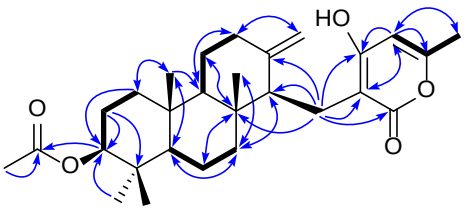


Fig 2 COSY (bold line) and selected HMBC (arrow line) correlation of aszonapyrone A (**I**).

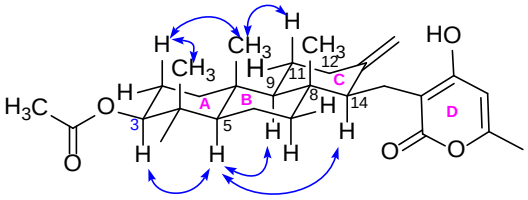


Fig 3 NOESY correlation of aszonapyrone A (**I**).

Compound (**II**) was obtained as colorless crystals, mp 173-175 ^{o}C. The ^{1}H and ^{13}C NMR spectral data of compound **II** (Table 1) were similar to those of the aszonapyrone A (**I**), except for the acetoxy group on **I** was replaced by a hydroxyl group. This indicated by NMR resonances of **I** at δ_H 2.01/δ_C 21. (C-2") and δ_C 171.7 (C-1") and IR absorption at 1728 cm^{-1} (C=O), while NMR signals of **II** were at δ_H 4.42/δ_C 81.1 for C-3. Therefore, compound **II** was identified as aszonapyrone B (Kanokmedhakul el al., 2011).

The results of cytotoxicity test of aszonapyrones A (**I**) and B (**I**) are showed that only aszonapyrone A (**I**) exhibited cytotoxicity against two cancer cell lines, KB and NCI-H187, with IC_{50} values of 48.8 and 4.62 µg/ml, respectively.

Table 1 ^{1}H and ^{13}C NMR data of aszonapyrone A (**I**) and aszopyrone B **(II)**

Position	^{1}H NMR		^{13}C NMR	
	I	**II**	**I**	**II**
1	1.64, m; 1.02, m	1.69, m; 0.98, m	38.1	38.6
2	1.59,m	1.56, m	23.6	26.8
3	4.42, t (8.2)	3.14, t (8.2)	81.4	78.4
4			37.6	38.7
5	1.07, m	0.83, m	59.7	55.3
6	1.57, m; 1.39, m	1.60, m	18.6	18.6
7	1.86, m; 1.42, m	1.89, m; 1.41, m	39.9	40
8			37.4	39.9
9	0.91, m	1.07, m	55.2	59.9
10			39.9	37.4
11	1.28, m	1.66, m, 1.29, m	23.4	23.3
12	2.25, m; 1.91, m	2.25, m; 1.89, m	38.1	38.1
13			148.9	148.9
14	2.43, m	2.45, m	53.5	53.4
15	2.57, m; 2.47, m	2.59, m; 2.46, m	18.7	18.7
16	4.82, s; 4.60, s	4.82, s; 4.61, s	106.1	105.8
17	0.71, s	0.74,s	14.8	14.7
18	0.80,s	0.81, s	16.2	15.9
19	0.79, s	0.94, s	16.1	27.6
20	0.81, s	0.74, s	27.8	15
2'			167.1	167.3
3'			102.7	102.7
4'			165.7	165.9
5'	5.82, s	5.87, s	100.5	100.5
6'			159.5	159.6
7'	2.10, s	2.14, s	19.2	18.9
1'			171.7	
2"	2.01, s		21	

Conclusions

Two meroterpoids were isolated from the crude EtOAc extract of the fungal biomass of *N. tatenoi*. Their structures were identified by spectroscopic method as aszonapyrone A (**I**) and aszonapyrone B (**II**). The bioactivity assay found that only aszonapyrone A (**I**) exhibited cytotoxicity against two cancers cell lines, KB and NCI-H187, with IC_{50} values 48.18 and 4.62 µg/ml, respectively.

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References

Eamvijarm, A., Gomes, N., Dethoup, T., Buaruang, J., Manoch, L., Silva, A., and Pedro, M. 2013. Bioactive meroditerpenes and indole alkaloids from the soil fungus *Neosartorya fischeri* (KUFC 6344), and the marine-derived fungi *Neosartorya laciniosa* (KUFC 7896) and *Neosartorya tsunodae* (KUFC 9213). Tetrahedron. 69 (5): 8583-8591

Jayasuriya, H., Zink, D., asilio, A., Vicente, F., Collado, J., Bills, G., Goldman, ML., Motyl, M. 2009. Discovery and antibacterial activity of glabramycin A-C from *Neosartorya glabra* by an antisense strategy. J. Antibiot. 62(5): 265-269.

Kanokmedhakul, K., Kanokmedhaku, S., Suwannatrai, R., Soytong, K., Prabpai, S., Kongsaeree, P. 2011. Bioactive meroterpenoids and alkaloids from the fungus *Eurotium chevalier*. Tetrahedron. 67: 5461-5468.

Kimura, Y., Hamasaki, T., Isogai, A., Nakajima, H. 1982. Structure of aszonapyrone A, a new metabolite produced by *Aspergillus zonatus*. Agric. Biol.chem. 46 (7): 1963-1965.

Masi, M., Andolfi, A., Veroique, MV., Boari, A., Cimmino, A., and Banuls, LM. 2013. Fischerindoline, a pyrroloindole sesquiterpenoid isolated from *Neosartorya pseudofischeri*, with in vitro growth inhibitory activity in human cancer cell lines. Tetrahedron. 69: 7466-7470.

Samson, RA., Hong, S., Peterson, SW., Frisvad, JC., Varga, J. 2007. Polyphasic taxonomy of *Aspergillus* section Fumigati and its teleomorph *Neosartorya*. Mycologia. 59: 147–203.