

Poster:

## Isolation and characterization of antimicrobial constituents from a terrestrial *Streptomyces* sp.: A preliminary result

Muhammad Bahi

Department of Organic Chemistry, Faculty of Mathematics and Natural Sciences  
Syiah Kuala University, Darussalam, 23111 Indonesia. Email: [bahi.usk@gmail.com](mailto:bahi.usk@gmail.com)

**Abstract.** Five phenolic derivatives, namely 2-methoxybenzene-1,3-diol (1), 2,3-dihydroxybenzamide (2), benadrostin (4), 2,3-dihydroxybenzoic acid (5) and tyrosol (6) were isolated from the terrestrial *Streptomyces* sp. Ank75. Three of them are new natural products. A further new antifungal is an azoxy derivative 3. All structures were elucidated based on spectroscopic and MS data and will be discussed here.

Keywords: Fermentation, algae, *Streptomyces* sp, derivatives

### Introduction

The genus of *Streptomyces* has been reported as the largest source of novel antibiotics from bacteria since the first report of streptomycin in 1942. Currently, many microbial antibiotics have been reported from genus *Streptomyces*, and the number of microbial antibiotics has increased exponentially for the last two decades (Watve *et al.*, 2001). In 2001, more than 70% of antibiotics had been isolated from genus *Streptomyces*, and 4% of them were used clinically as human drugs (Clardy *et al.*, 2006).

In this research, we reported the secondary metabolites isolated from terrestrial *Streptomyces* sp. Ank75. One of the secondary metabolites from the strain Ank75 was an azoxy derivative, compound **3**, which was also active as antifungal. In addition, five phenolic compounds, namely 2-methoxybenzene-1,3-diol (**1**), 2,3-dihydroxybenzamide (**2**), benadrostin (**4**), 2,3-dihydroxybenzoic acid (**5**) and tyrosol (**6**) were isolated from the terrestrial *Streptomyces* sp. Ank75 (Figure 1). Due to the complexity of the structure, only compound **3** will be discussed in more detail here.

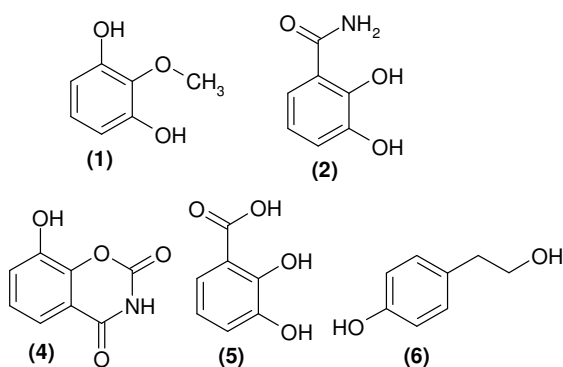


Figure 1: Isolated compounds from *Streptomyces* sp. Ank75

### Materials and Methods

#### Isolation and bioassay

The *Streptomyces* sp. Ank75 strain was fermented in 25 L scale on M<sub>2</sub> medium for 5 days at 28 °C. The culture broth was filtered over Celite and the water phase was adsorbed on resin Amberlite XAD-16 column and eluted with methanol after washing with demineralised water prior to the elution with methanol. The biomass was exhaustively extracted with ethyl acetate and acetone. TLC of crude extract from each organic residues was identical, thus the three extracts (6.0 g) were combined together for further work-up procedure (Figure 2).

**Poster:**

The separation of crude extract (6.0 g) was performed by silica gel column chromatography (3 × 75 cm, 200 g) using aCH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH gradient. Based on the spot pattern on the TLC, four fractions were selected for further purification. The 2-methoxybenzene-1,3-diol (**1**) was obtained from fraction II *via* Sephadex LH-20 column using CH<sub>3</sub>OH as a solvent. From fraction III, benadrostin (**4**) was isolated *via* Sephadex LH-20 column using CH<sub>3</sub>OH as a mobile phase. Compounds **2**, **3**, **5** and **6** were isolated from fraction IV after being subjected to Sephadex LH-20 (MeOH) and RP-18 (MeOH:H<sub>2</sub>O) columns, respectively. The isolation procedure from crude extract of *Streptomyces* sp. Ank75 was shown in Figure 2.

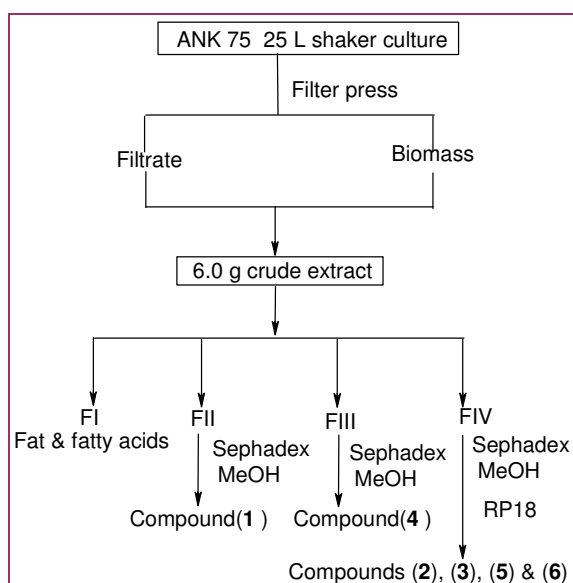


Figure 2: Work up scheme of *Streptomyces* sp. Ank75

**Biological activity**

The terrestrial *Streptomyces* sp. Ank75 formed on M<sub>2</sub> agar medium a white aerial mycelium within 3 days at 28 °C. The crude extract of the strain showed activities against *Candida albicans*, *Mucor miehei* (Tü 284), *Chlorella vulgaris*, *Chlorella sorokiniana*, but was inactive against bacteria and *Artemia salina* (brine shrimp test) (Table 1).

Table 1: Anti-microbial activity of the crude extract *Streptomyces* sp. Ank75 (1 mg/ml, diameter of inhibition zones in mm).

Tested microorganisms	Inhibition zone Ø [mm]
<i>Candida albicans</i>	15
<i>Mucor miehei</i> (Tü 284)	25
<i>Chlorella vulgaris</i>	33
<i>Chlorella sorokiniana</i>	31
<i>Artemia salina</i>	0%

**Result and Discussion**

ESIMS of **3** afforded 255.2 ([M<sup>+</sup> Na]<sup>+</sup>, 100), and HRESI MS delivered the formula C<sub>10</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>. One of the two double bond equivalents is due to a CO group, the other one belongs to a ring, as double bond signals are missing. Three alternative structures A, B or C were taken into account, where A and C are azoxy derivatives, and B is a highly strained oxadiaziridine (Figure 3). According to the literature, structure B is thermally labile and less stable than structures A and C (Hecht and Greene, 1967; Swigert and Taylor, 1971). Structure C is a tautomer of an instable primary nitrosamine. The <sup>13</sup>C NMR spectrum showed an sp<sup>3</sup> signal at δ<sub>C</sub> 56.1 for a carbon, which is possibly attached to nitrogen. Kariya *et al.* (2006) have reported a related compound bearing an azoxy moiety, which corresponds to structures A or C.

Poster:

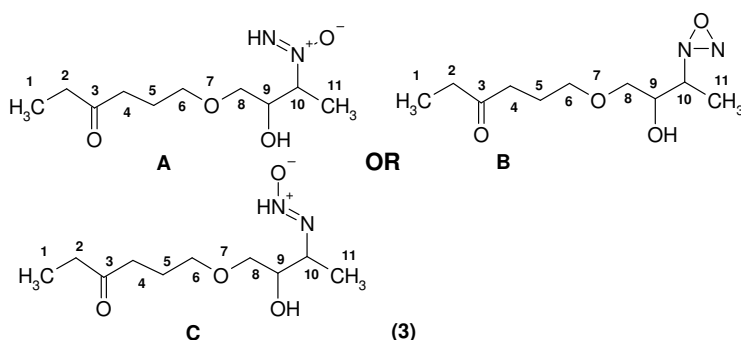


Figure 3: Plausible structures of compound **3**.

## Conclusions

To sum up, five phenolic and one azoxy derivatives have been isolated from the terrestrial *Streptomyces* sp. Ank75. In addition, compound **3** (azoxy derivative) has also shown antifungal activity in this research.

## Acknowledgements

MB would like to thank Dr .H. Frauendorf and Mr. R. Machinek for the mass and NMR measurements, H. Laatsch, H. Anke and H. Kohl for supervision, collaboration and technical assistance, and the DAAD for financial support during doctoral study in Germany.

## References

- Clardy, J.; Fishbach, M. A.; Walsh, C. T. 2006. New antibiotics from bacterial *natural* products. *Nature Biotechnol*, 24: 1541-155.
- Hecht, S. St., Greene, F. D. 1967. Di-tert-butyloxadiaziridine, the cyclic form of an azoxy group. Ring-chain isomerism in three-membered rings. *JACS* 89: 6761-6762.
- Kariya, Y., Kubota, T., Fromont, J., & Kobayashi, J. I. Pynadine A, a novel pyridine alkaloid with an azoxy moiety from sponge *Cribrochalina* sp. *Tetrahedron Lett.*, 2006, 47, 997-998.
- Swigert, J., & Taylor, K. G. 1971. Aliphatic azoxy compounds. I. Photolytic isomerization of azoxyalkanes. *JACS*, 93: 7337-7338.
- Watve, M. G.; Tickoo, R.; Jog, M. M.; Bhole, B. D. 2001. How many antibiotics are produced by the genus *Streptomyces*?. *Arch. Microbiol.*, 176: 386-390.