Experiments
Експерименти в природните науки

A NEW APPROACH FOR THE SYNTHESIS OF P-ANISYL ETHYL FUMARATE: A C-9154 ANTIBIOTIC ANALOGUE

Maulidan Firdaus, Ahmad Ainurofiq, Aulia Vellarani
Sebelas Maret University – Indonesia

Abstract. A simple, energy-efficient, and relatively quick synthetic procedure for the synthesis of *p*-anisyl ethyl fumarate from *p*-anisaldehyde has been carried out. The synthesis was started by solvent-free reduction of the aldehyde using sodium borohydrate. The second step was solvent-free condensation of the resulting alcohol with maleic anhydride to be followed by esterification of the resulting acid with ethanol in the presence of benzenesulfonic acid as the catalyst. Satisfactorily isolated yields (65-98%) were achieved confirming that the preparation of the C-9154 antibiotic analogue is possible in sustainable fashion.

Keywords: *p*-anisaldehyde, *p*-anisyl ethyl fumarate, C-9154 antibiotic, solvent-free

Introduction

Reduction microbes that are resistant to most antibiotics in the market today have a concern some researchers to synthesize new antibiotic compounds. One of the types of antibiotics that have potential to be developed is a C-9154 antibiotic (**1**). C-9154 (Fig. 1) is a class of antibiotic isolated from *Streptomyces ishigakiensis* by Hasegawa in 1975 through fermentation process (Hasegawa et al., 1975). Although the yield obtained *via* this process is extremely low (ca 0.02%), this compound exhibited a wide activity against gram-negative and gram-positive bacteria, i.e., *Staphylococcus aureus* and *Escherichia coli* with minimum inhibition concentration (MIC) varied from 10-100 μg/mL and LD_{50} in mice of 75 mg/kg (Hasegawa et al., 1975). The low yield of **1** by fermentation has led some researchers to synthesize derivatives of antibiotic **1**. Several strategies have been developed and reported by Jumina who has managed to synthesize several derivatives **1** from vanillin, benzyl chloride, furfural, salicyl alcohol, anisyl alcohol, benzilamina, and aniline (Jumina et al., 2001; 2002; 2005). Antibacterial activity against the synthesized compounds have also been conducted, and the results showed that some of these compounds proved to be active and effective as an antibacterial. Attracted by the

high activity of antibiotics **1** and its derivatives, recently, Bello et al. (2012; 2013) have successfully synthesized several derivatives of **1**. Antifungal and antibiotic activity test against these derivatives have been reported, and the results showed that the biological activity of several compounds that have been synthesized against clinical isolates of *Aspergillus nigre* and *Candida albicans* proven effective as an antibiotic and/or antifungal, and even gave better result compared to the antibiotic and antifungal compounds on the market.

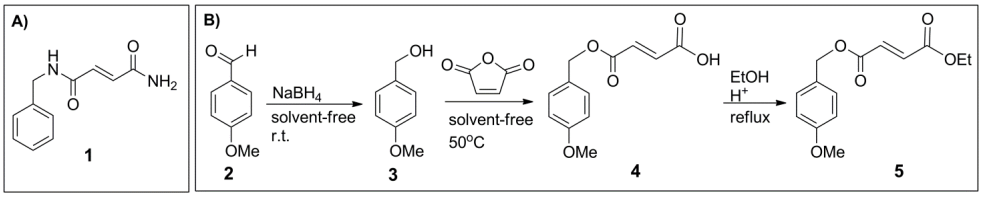

Fig. 1. Structure of **1** (A) and synthetic scheme of **5** (B)

Although several compounds derived from **1** have been successfully synthesized and proved to be active as antibacterial and antifungal, it is unfortunate that the synthesized method is not environmentally friendly and not in accordance with the principles of green chemistry. The experiment methods used to synthesize the compound are not "green". Some used organic solvent, created excess waste, and consumed energy on its processes. Many organic solvent, which are ecologically harmful, and their use should therefore be minimized as far as possible or even avoided altogether.

One of C-9154 antibiotic analogues that has prospective to be developed is *p*-anisyl ethyl fumarate (**5**). This compound has been reported actively inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* with MIC value of 15 μg/mL for both bacteria (Jumina et al., 2005). Attracted to the simplicity of the structure and the economic value of the antibiotic product derived from **1** in the field of pharmacology, as well as considering aspects of health and ecological environment of generated waste, it is necessary to perform the solvent-free synthesis of antibacterial compound analogue of **1** (Fig. 1), i.e, **5**, from *p*-anisaldehyde (**2**).

Materials and methods

Reagents and analysis

Unless stated otherwise, all chemicals used in this research were commercial products of high purity purchased from Merck. All solvents (technical grade) were purchased and underwent without further purification.

Thin layer chromatography (TLC) was carried out on silica gel TLC-cards (layer thickness 0.20 mm, Merck). Permanganate reagent was applied as developing solution. Analytical GC-MS characterization was performed with a Shimadzhu QP

5000 spectrometer using flame ionization detection. FTIR spectra were recorded on Shimadzu FTIR 8201 spectrophotometer.

Experimental procedures

*Solvent free reduction of **2** with $NaBH_4$*

$NaBH_4$ (0.01 mol) was carefully and finely ground with a mortar and pestle. Aldehyde **2** (0.01 mol) was then added and grinding of the reaction mixture was continued at room temperature for 10 minutes. The reaction was stopped using a solution of saturated $NaHCO_3$. The mixture was then extracted with dichloromethane (3 x 10 mL). The organic layer was dried with Na_2SO_4 anhydrous to be followed with evaporation of the solvent to give **3** (98%) as a light yellow oil. The product was characterized by FTIR and GC-MS. $FTIR_{vmax}$ (neat) cm^{-1}: 3361 (O-H), 3002, 1615, and 1516 (ArH), 3000 – 2800 (-CH_3), 1456 (-CH_2), 1033 (-OCH_3). MS (m/z): 138 (M^+), 121 (M-17), 109 (M-28). These spectral data are in a full accordance with the reported literature (Jumina et al., 2005).

*Synthesis of **5***

A mixture of maleic anhydride (10 mmol) and **3** (10 mmol) was carefully stirred at 50 °C. The reaction was observed by TLC (acetone / ethanol 1:1; SiO_2). The resulting product was allowed to cool, added with ethyl acetate (30 mL), and washed with H_2O (3x50 mL). The organic phase was dried with anhydrous sodium sulphate and evaporated. Product identification was carried out by FTIR and GC-MS. This product (**4**) was then used for the next reaction without any purification. $FTIR_{vmax}$ (KBr) cm^{-1}: 3447 (O-H), 3002, 1615, and 1516 (ArH), 3000 – 2800 (-CH_3), 1716 & 1733 (C=O), 1615 (C=C) 1461 (-CH_2), 1033 (-OCH_3). MS (m/z): 137 (M-99), 121 (M-115), 109 (M-127).

Esterification of **4** was then performed according to the reported literature (Jumina et al., 2005) with a slight modification. A Mixture of **4** (5 mmol), ethanol (15 mL), and benzenesulfonic acid (ca 0.3 g) was stirred at 78 °C for 3 h. The reaction mixture was allowed to cool to be followed by solvent evaporation. The filtrate was dissolved with H_2O (30 mL) and extracted with dichloromethane (3x30 mL). The organic layer was washed with H_2O (2x50 mL), dried with Na_2SO_4 anhydrous and evaporated to colorless oil. This oil was passed through a suction chromatography column (silica) eluted with dichloromethane/petroleum ether (4:1) to yield **5** (65%) as a colourless liquid. Product was identified by FTIR and GC-MS. $FTIR_{vmax}$ (neat) cm^{-1}: 3002, 1613, and 1514 (ArH), 3000 – 2800 (-CH_3), 1717 & 1733 (C=O), 1612 (C=C), 1464 (-CH_2), 1033 (-OCH_3). MS (m/z): 166 (M-98), 137 (M-127) 121 (M-143), 109 (M-155). These spectral data are in a full agreement with the reported literature (Jumina et al., 2005).

Result and discussion

Although compound of **5** was already synthesized by Jumina *et al*. (2005), the reported method was less efficient and not environmentally benign since it was using solvent and took quite a lot of energy and reaction time in the reflux process. In this research, synthesis of **5** was started by reduction of **2** using $NaBH_4$. Aldehyde **2** itself was chosen as starting material since this compound is a renewable raw material, an alternative for substituting petroleum based material. It can be obtained by oxidation of anethole (Xiao et al., 2008, Alvarez et al., 2006) the major component of anise oil [ca. 60–80%] (Budavari, 1996). According to the principles of green chemistry, a chemical reaction is preferably carried out using a safer solvent, or whenever possible should be done without solvents (Anastas & Warner, 2000). The reaction was done by grinding the aldehyde with $NaBH_4$ using a mortar and pestle under solvent-free condition at room temperature for 10 minutes to yield alcohol **3**. This method presents an enhancement in the efficiency of resource utilization, reducing energy usage and solvent waste, and accelerating reaction time (Cho et al., 2006). FTIR and GC-MS analyses of the product revealed the successful of the reduction. FTIR spectra clearly shows no absorption of carbonyl aldehyde group around 1700 cm^{-1}. The appearance of absorption peak at 3361 cm^{-1} indicates the presence of hydroxyl group of alcohol (Fig. 2). MS spectra of this alcohol gave ion molecular peak at m/z = 138 which is in accordance with the molecular weight of **3**.

The second step of the synthesis was done by condensation reaction of **3** with maleic anhydride. This reaction was already reported by Jumina et al. (2005) with benzene as the solvent at 90 °C for 2.5 hours. Unfortunately, the reported method is still not sustainable and not in accordance with the principles of green chemistry. Benzene is toxic and carcinogenic compound (Aksoy, 1989). Furthermore the reaction process also requires energy to reflux. In this study, innovation technique has been carried out by reacting **3** and maleic anhydride without the use of solvent. Initially the reaction was performed at room temperature. TLC analysis showed that the spot of starting material was not completely disappeared. Further attempt was done by performing the reaction at 50 °C. The results of TLC analysis showed that the spot of the starting material had disappeared and new spot appeared. FTIR spectra (Fig. 2) showed absorption of OH carboxylic acid groups at 3447 cm^{-1}, the appearance of C=O group at 1733 and 1716 cm^{-1}, and C=C group at 1615 and 1516 cm^{-1} provide sufficient evidence for the formation of acid **4**. Although the MS spectra of this product did not reveal molecular ion peak at the estimated value, the fragmentation pattern showing peaks at m/z 137, 121 (base peak), and 91 provided more evidence for the formation of **4** (Jumina et al., 2005).

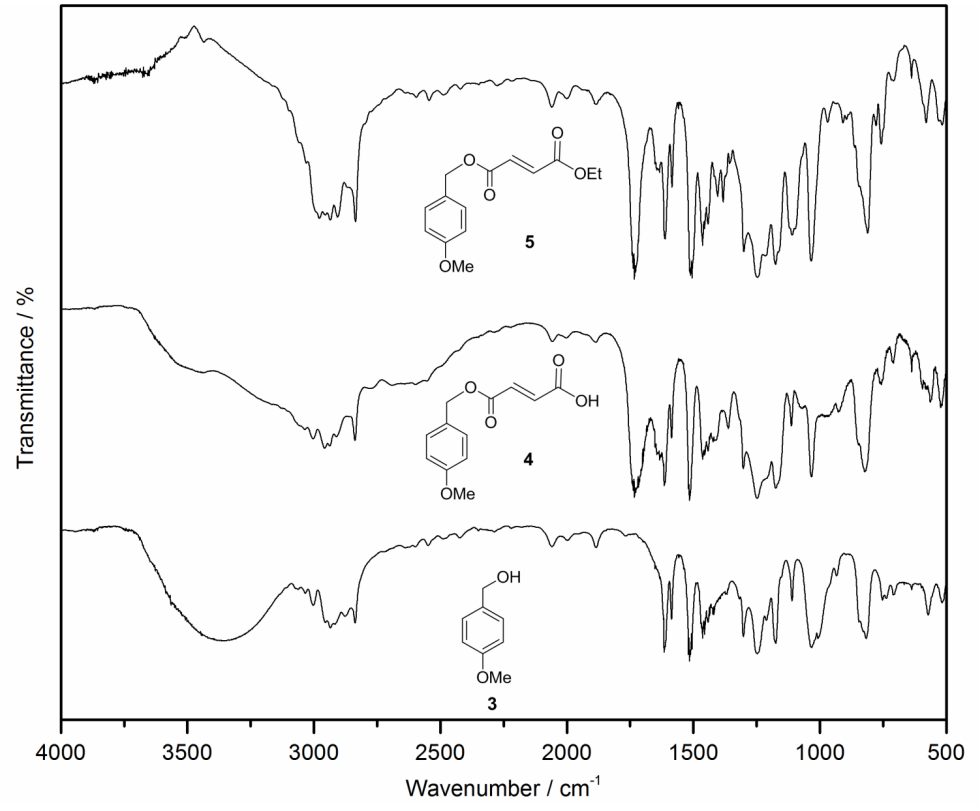

Fig. 2. FTIR spectra of **3** (bottom), **4** (middle) and **5** (top)

The last step of the synthesis was performed by esterification of **4** with ethanol in the presence of benzenesulfonic acid at 78 °C for 3 h. FTIR and GC-MS analysis revealed that the esterification was successful. The disappearance of absorption at 3447 cm^{-1} from OH carboxylic acid group strongly showed that the result of the reaction was **5**. Identification using mass spectra indicated that the product of this reaction was **5**. This was shown by the fragmentation pattern displaying the peaks at m/z 166, 137, 121 (base peak), and 109 that gave adequate proof for the formation of **5** (Jumina et al., 2005).

Overall, in comparison with conventional techniques, solvent-free reduction and condensation is more feasible than solution phase methodology in term of simplicity, efficiency energy, and reaction time. Some of green chemistry principles (Anastas & Warner, 2000) have been applied i.e. minimize waste and maximize energy saving; minimize use of solvents and toxic reactants; use catalyst and efficient chemical reactions; utilize renewable feed stocks in a sustainable approach.

Conclusion

We present a versatile and efficient way to synthesize C-9154 antibiotic analogue **5** from renewable aldehyde **2**. Compared with conventional methods, present procedures have the advantages in shorter reaction time, simplicity, and efficiency energy.

Acknowledgement. The authors would like to thank Sebelas Maret University for financial support in the form of "Hibah Doktor Baru" project.

REFERENCES

Aksoy, M. (1989). Hematotoxicity and carcinogenicity of benzene. *Environ. Health Persp*., *82,* 193 – 197.

Alvarez, H.M., Barbosa, D.P., Fricks, A.T., Aranda, D.A.G., Valdés, R.H. & Antunes, O.A.C. (2006). Production of piperonal, vanillin, and *p*-anisaldehyde via solventless supported iodobenzene diacetate oxidation of isosafrol, isoeugenol, and anethol under microwave irradiation. *Org. Process Res. & Dev., 10*, 941 – 943.

Anastas, P.T. & Warner, J.C. (2000). *Green chemistry: theory and practice*. Boston: Oxford University Press.

Bello, I.A., Ndukwe, G.I., Amupitan, J.O., Ayo, R.G. & Shode, F.O. (2012). Syntheses and biological activity of some derivatives of C-9154 antibiotic. *Int. J. Med. Chem., 2012*, art. no. 148235.

Bello, I.A., Ndukwe, G.I., Amupitan, J.O., Ayo, R.G., Shode, F.O. & Bello, E.O. (2013). Antifungal ptential of C-9154 derivatives against *Candida albicans* and *Aspergillus nigre (pp. 258 – 268). In: Méndez-Vilas (Ed.). Microbial pathogens and strategies for combating them: science, technology and education*. *Badajoz: Formatex Research Center.*

Budavari, S. (1996). The Merck index, an encyclopedia of chemicals, drugs, and biological. Rahway: Merck & Co Inc.

Cho, B.T., Kang, S.K., Kim, M.S., Ryu, S.R. & An, D.K. (2006). Solvent-free reduction of aldehydes and ketones using solid acid-activated sodium borohydride. *Tetrahedron*, *62*, 8164 – 8168.

Hasegawa, T., Asai, M., Haibara, K., Yamano, T., Iwasaki, H. & Yoneda, M. (1975). A new antibiotic, C-9154. *J. Antibiotics*, *28*, 713 – 716.

Jumina, Siswanta, D. & Zulkarnain, A.K. (2001). Synthesis and antibacterial activity of C-9154 derivatives from vanillin. *Indonesian J. Pharm., 12*, 85 – 91.

Jumina, Tahir, I. & Zulkarnain, A.K. (2002). Synthesis and antimicrobial activity evaluation of ethyl salicyl fumarate and ethyl furfuryl fumarate. *Indonesian J. Pharm., 13*, 207 – 214.

Jumina, Zulkarnain, A.K. & Mulyono, P. (2005). Preparation and antibacterial activity of *p*-anisyl ethyl fumarate and ethyl *n*-phenyl fumaramate, *Indonesian J. Pharm*., *16*, 116 – 123.

Xiao, Y., Huang, H., Yin, D., Guo, D., Mao, L. & Fu, Z. (2008). Oxidation of anethole with hydrogen peroxide catalyzed by oxovanadium aromatic carboxylate complexes. *Cat. Commn., 10*, 29 – 32.

✉ **Dr. Maulidan Firdaus (corresponding author)**
Department of Chemistry
Faculty of Mathematics and Natural Sciences
Sebelas Maret University
Jl. Ir. Sutami 36A Surakarta
57126 Indonesia
E-mail: maulidan.firdaus@staff.uns.ac.id