Majalah Farmasi Indonesia, 13(4), 207-214, 2002

SYNTHESIS AND ANTIMICROBIAL ACTIVITY EVALUATION OF ETHYL SALICYL FUMARATE AND ETHYL FURFURYL FUMARATE

SINTESIS DAN UJI KHASIAT ANTIMIKROBA TERHADAP ETIL SALISIL FUMARAT DAN ETIL FURFURIL FUMARAT

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ABSTRACT

This research was conducted in order to synthesize and investigate the antimicrobial activity of ethyl salicyl fumarate and ethyl furfuryl fumarate. These two target molecules were chosen as the former is the derivative of C-9154 antibiotic containing phenolic hydroxyl group, whereas the latter is an example of C-9154 antibiotic derivative bearing furan ring instead of benzene.

Ethyl salicyl fumarate was synthesized from methyl salicylate through reduction with $LiAlH_4$, condensation of salicyl alcohol with maleic anhydride, and esterification of salicyl maleic acid with ethanol in the presence of benzenesulfonic acid as the catalyst. These reactions gave satisfactory yields (76-92 %) in all steps involved. Similar to this procedure, ethyl furfuryl fumarate was prepared from furfural through reduction with $NaBH_4$ followed by condensation of the resulted furfuryl alcohol with maleic anhydride and esterification of furfuryl maleic acid with ethanol in the presence of benzenesulfonic acid. Although the reduction of furfural and the condensation of furfuryl alcohol with maleic anhydride could be performed smoothly, the esterification of furfuryl maleic acid with ethanol only gave 38 % yield of ethyl furfuryl fumarate.

The results of antimicrobial activity test showed that the value of minimum inhibition concentration (MIC) of salicyl maleic acid and ethyl salicyl fumarate towards *Staphyllococcus aureus* were 500 and 100 μg/mL, whereas towards *Eschericia coli* were 2000 and 4000 μg/mL respectively. In contrast, the MIC values of furfuryl maleic acid and ethyl furfuryl fumarate towards *Staphyllococcus aureus* and *Eschericia coli* were 150 and 100 μg/mL respectively.

Keywords: *synthesis, activity, C-9154 antibiotic, fumarate.*

ABSTRAK

Penelitian ini dilakukan dengan tujuan melakukan sintesis dan uji khasiat antimikroba terhadap etil salisil fumarat dan etil furfuril fumarat. Kedua molekul target tersebut dipilih dengan pertimbangan bahwa etil salisil fumarat akan merupakan contoh turunan antibiotik C-9154 yang mengandung gugus fenol, sedangkan etil furfuril fumarat akan menjadi contoh turunan antibiotik C-9154 yang mengikat cincin furan.

Etil salisil fumarat disintesis dari metil salisilat melalui reduksi dengan $LiAlH_4$, kondensasi dari salisil alkohol dengan anhidrida maleat, dan esterifikasi asam salisil maleat dengan etanol menggunakan katalis asam benzenasulfonat. Keseluruhan tahapan reaksi tersebut memberikan rendemen hasil yang

memuaskan (76-92 %). Serupa dengan itu, etil furfuril fumarat disintesis dari furfural melalui reduksi menggunakan $NaBH_4$ diteruskan dengan kondensasi furfuril alkohol dengan anhidrida maleat dan esterifikasi asam furfuril maleat dengan etanol. Walaupun reduksi furfural dan kondensasi furfuril alkohol dengan anhidrida maleat dapat dilakukan dengan baik, esterifikasi asam furfuril maleat dengan etanol hanya menghasilkan 38 % etil furfuril fumarat.

Hasil uji khasiat antimikroba menunjukkan bahwa harga konsentrasi hambatan minimum (MIC) dari asam salisil maleat dan etil salisil fumarat terhadap *Staphyllococcus aureus* masing-masing adalah 500 dan 100 μg/mL, sedangkan terhadap *Eschericia coli* adalah 2000 and 4000 μg/mL. Sebaliknya, harga MIC dari asam furfuril maleat dan etil furfuril fumarat terhadap *Staphyllococcus aureus* dan *Eschericia coli* masing-masing adalah 150 dan 100 μg/mL.

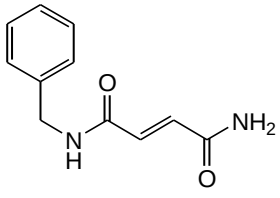
Kata kunci: *sintesis, aktivitas, antibiotik C-9154, fumarat.*

INTRODUCTION

Maleic acid (cis) and fumaric acid (trans) are two among the known compounds which have been used in the field of pharmacy, medicine, and agriculture since a long time ago. Specifically, the derivatives of these two isomeric compounds have been utilized for the preparation of antibiotics, antifungis, antihistamins and plant growth regulators (Stecher, 1968). Just to mention some examples are the ester form of melanilic acid which has been patented as an antifungi (Ligett, 1959), A-19009 antibiotic discovered by Molloy (1972), fumaryl DL-alanin antibiotic (Birkingshaw, 1942), and maleic hydrazides which are known to be able to inhibit the growth of several horticulture plants (Schoene, 1949). In addition, combination of maleic acid and chlorpheniramine known as chlorpheniramine maleate is commonly found in some cough formulaes such as Benadryl, Formula 44 and Alerin.

Another active compound which also contains fumaric fragment is C-9154 antibiotic. C-9154 **(1)** is a class of antibiotic isolated by Hasegawa (1975) from *Streptomyces ishigakiensis* through a fermentation process. The compound showed a broad spectral activity against Gram-positive and Gram-negative bacteria such as *Escherichia coli* and *Staphylococcus aureus* with minimum inhibition concentration (MIC) varied from 10-100 μg/mL and LD_{50} in mice of 75 mg/kg. The chemical structure of C-9154 antibiotic itself basically consists of two fragments i.e. phenylacetic acid and fumaramide.

Recently we have developed a new strategy for the synthesis of C-9154 antibiotic derivatives from vanillin (Jumina dkk., 2000 and 2001). Within this strategy, vanillin was converted to 4-ethoxy-3-methoxybenzylaniline in three stages. This benzylaniline was then reacted with maleic anhydride to be followed by esterification of the related acid with absolute ethanol in the presence of H_2SO_4. This method is very efficient and satisfactory yields (70-95%) were obtained in each step. The biological activity of both C-9154 derivatives obtained **(2** and **3)** have already been investigated in which the MIC of **2** towards *Staphyllococcus aureus* and *Escherichia coli* were 2500-3000 μg/mL, whereas that of **3** towards the same bacteria were 500-1000 μg/mL. Thus, conversion of the carboxylic acid group in **2** to an ester functionality in **3** has led to an increase of anti microbe activity to approximately four times.



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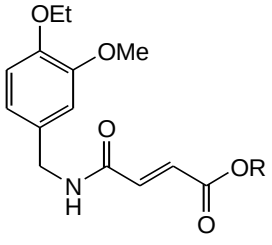
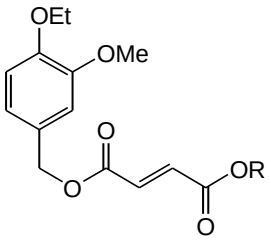


2 (R = H)
3 (R = Et)



4 (R = H)
5 (R = Et)

The above method has also been extended on the case of 4-ethoxy-3-methoxybenzyl alcohol simply prepared via reduction of ethyl vanillin with $NaBH_4$. This type of benzyl alcohol derivatives was reacted with

maleic anhydride to be followed by treatment with absolute ethanol in the presence of H_2SO_4. Again, excellent yields (78-93 %) were achieved in all steps. The acid form **(4)** of the derivative obtained still gave a weak anti microbe effect (MIC 2000-2500 μg/mL) towards *Staphyllococcus aureus* and *Escherichia coli*. However, a significant anti microbe activity towards those two microbes (MIC 400-700 μg/mL) were observed on the case of the ester derivative **(5)**.

In order to obtain a more potent C-9154 antibiotic derivatives, it was of interest to synthesize C-9154 antibiotic derivatives bearing phenolic hydroxyl group and furfuryl moiety. Regarding the former, this should be more polar than compound **2**, **3**, **4**, and **5** previously obtained. Whereas regarding the latter, this derivative would have a furan ring instead of benzene ring as seen in the structure of compound **2**, **3**, **4**, and **5**. These two target compounds are planned to be prepared respectively from methyl salicylate, the main constituent of oil of wintergreen, and furfural.

METHODOLOGY

Chemicals

All chemicals used in this research were reagent grade from Merck. **Apparatus.** The equipment used in this experiment involved JEOL MY60 proton NMR spectrometer, Shimadzu FTIR 8201 PC spectrophotometer, and Shimadzu QP 5000 Gas Chromatograph-Mass Spectrometer.

Reduction of methyl salicylate

A solution of methyl salicylate (1.4 g, 9.22 mmol) in dry dioxane (15 mL) was heated at 60^{o}C in a round bottomed flask equipped with condenser. Into this solution was then added slowly lithium aluminium hydride (1.06 g, 27.66 mmol) through the condenser. After all $LiAlH_4$ has been added, the mixture was heated at 90-95^{o}C for 2 h. The resulted mixture was allowed to cool to room temperature, then cold water (5 mL) was added slowly to destroy excess $LiAlH_4$. The mixture was acidified using HCl 10%, then this was extracted with ethyl acetate (3x40 mL). The combined organic layers were washed with water (70 mL), dried over anhydrous sodium sulfate and evaporated to leave salicyl alcohol (0.87 g, 76%) which was found as colorless sticky oil. Identification of this product was carried out by means of IR and proton NMR spectrometers.

Condensation of salicyl alcohol with maleic anhydride

A solution of salicyl alcohol (2.0 g, 16.13 mmol) in dioxane (15 mL) was added dropwise into a solution of maleic anhydride (1.90 g, 19.39 mmol) in dioxane (15 mL). The mixture was stirred at 70-80^{o}C for 3.5 h. The resulted mixture was allowed to cool, then diluted with ethyl acetate (50 mL). The mixture was washed with water (3x70 mL) and the organic layer was dried over anhydrous sodium sulfate and evaporated to leave sticky light yellow oil of salicyl maleic acid (3.3 g, 92 %). This product was characterized by means of IR spectrometer.

Synthesis of ethyl salicyl fumarate

A mixture of salicyl maleic acid (1.0 g, 4.50 mmol), absolute ethanol (10 mL), and benzene sulfonic acid (ca. 0.3 g) was stirred and heated at reflux for 3.5 h. The resulted mixture was allowed to cool, then the solvent was removed by means of rotary evaporator. The residue was diluted with water (40 mL), then extracted with ethyl acetate (3x40 mL). The combined organic layers were washed with water (2x70 mL), dried over anhydrous sodium sulfate and evaporated to afford the desired ester (0.86 g, 76 %) which was found as light yellow oil. This product was characterized by means of IR and proton NMR spectrometers.

Reduction of furfural

Into a solution of furfural (4.60 g, 48.14 mmol) in absolute ethanol (30 mL) was added sodium borohydride (3.64 g, 96.29 mmol). The mixture was heated at reflux for 2 hours, then allowed to cool down and evaporated to dryness. The residue was diluted with water (50 mL), then extracted with ethyl acetate (3x30 mL). The combined organic layers were washed with water (2x80 mL), dried over anhydrous sodium sulfate and evaporated to give furfuryl alcohol (2.50 g, 53 %). This product was identified by means of IR and GC-MS spectrometers.

Condensation of furfuryl alcohol with maleic anhydride

A solution of maleic anhydride (1.75 g, 17.86 mmol) in benzene (15 mL) was stirred and warmed at 50^{o}C. Into this solution was added dropwise a solution of furfuryl alcohol (1.35 g, 13.81 mmol) in benzene (15 mL). The heating was continued for 2 hours, then the mixture was allowed to cool and diluted with ethyl acetate (70 mL). The mixture was washed with water (3x80 mL), dried over anhydrous sodium sulfate, and evaporated to afford furfurylmaleamic acid (1.95 g, 72 %) as a brown oil.

Synthesis of ethyl furfuryl fumarate

A mixture of furfuryl maleic acid (1.0 g, 4.50 mmol), absolute ethanol (10 mL), and benzene sulfonic acid (ca. 0.3 g) was stirred and heated at reflux for 3.5 h. The resulted mixture was allowed to cool, and further treatment according to procedure as described for the preparation of ethyl salicyl fumarate afforded ethyl furfuryl fumarate (0.38 g, 38 %) as a brown oil. The product was identified by means of IR spectrometer.

Determination of anti microbial activity of the derivatives synthesized

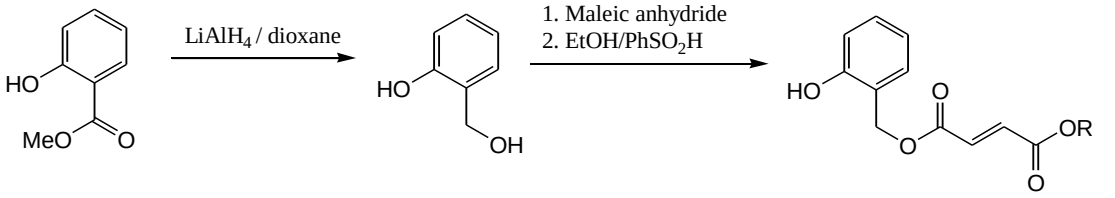
A series of solutions of each C-9154 derivative were prepared and placed in tubes containing *Staphyllococcus aureus* and *Escherichia coli*. The samples were incubated at 37^{o}C, and the inhibition effect of each sample was observed.

The C-9154 antibiotic derivatives obtained from the experiment were placed on petri dish containing Gram positive and negative bacteria. The samples were incubated at 37^{o}C using agar media and the inhibition effect of each sample was observed.

RESULTS AND DISCUSSION

Synthesis of ethyl salicyl fumarate

As stated previously, the preparation of ethyl salicyl fumarate was conducted in order to generate C-9154 antibiotic derivatives possessing phenolic hydroxyl group. It was of interest to investigate whether the existence of such free hydroxyl group would enhance its antimicrobe activity or not in comparison to those of compound **2**, **3**, **4**, and **5** previously synthesized. For this purpose, ethyl salicyl fumarate was prepared from methyl salicylate, the main constituent of oil of wintergreen, in 3 steps i.e. (1) reduction of methyl salicylate, (2) condensation of salicyl alcohol with maleic anhydride, and (3) esterification of salicyl maleic acid with ethanol (Scheme 1).



methyl salicylate *salicyl alcohol* *salicyl maleic acid (R = H; **6**)*
*ethyl salicyl fumarate (R = Et; **7**)*

Scheme 1

The reduction of methyl salicylate was conducted using $LiAlH_4$ in dry dioxane at reflux for 2 hours to give 82 % yield of the desired salicyl alcohol or *o*-hydroxybenzyl alcohol which was found as a white sticky solid. It is interesting to note in here that a safer procedure was found when methyl salicylate was added slowly to a hot (70-80^oC) suspension of $LiAlH_4$ in dioxane rather than heating slowly a mixture of $LiAlH_4$, methyl salicylate and dioxane. In this later procedure, a rather uncontrolled reaction occurred when the temperature reached at about 60^oC. Such a danger was not found at all when the former procedure was applied. In addition, it is worthy to mention that the resulted salicyl alcohol is best to be stored in a fridge. It was discovered that storing of the compound at room temperature for couple of days has changed it to be a waxy solid which was insoluble in most organic solvents such as dichloromethane, chloroform, ethyl acetate, dioxane, diethyl ether, benzene, toluene, and carbon disulfide.

The IR spectrum of the product showed the existence of two strong broad bands of OH groups at 3450 and 3163 cm^{-1}. The former is originated from CH_2OH, whereas the latter is from the phenolic OH group. The phenolic OH group appeared at a lower frequency compared to that of CH_2OH as the lone pair electron of the oxygen atom in phenol involves in delocalization. As expected, the spectrum did not show any absorption of C=O stretching frequencies which normally arise at around 1700 cm^{-1}. Therefore, the IR spectrum clearly proved that the C=O group of the starting material has been reduced to CH_2OH.

The proton NMR spectrum of the resulted salicyl alcohol displayed three signals, which indicated the existence of three types of protons. A downfield multiplet appearing at 6.7-7.2 ppm (4 H) is supposed to be originated from the resonance of the phenyl protons. The pattern of the peak, which is slightly irregular, may be attributed by the existence of two different substituents lying in an *ortho* orientation in the benzene ring. Singlets appearing at 4.8 ppm (1 H) and 3.7 ppm (2 H) respectively correspond to the resonance of OH and methylene protons. Although the spectrum is sufficiently clean, the integration of the phenyl proton is a bit less than the expected value.

Following the above success, the resulted salicyl alcohol was then reacted with maleic anhydride. The reaction was conducted in dioxane at 70-80^oC for 2 hours to give yellow sticky oil of salicyl maleic acid in 70% which could be viewed as the acid form of C-9154 antibiotic derivative. The IR spectrum of this product showed OH stretching frequency at 3331 cm^{-1}, carbonyl stretching frequency at 1778 cm^{-1}, and C=C stretching frequency at 1620-1593 cm^{-1}. The existence of only one OH absorption band rather than two as seen in the IR spectrum of salicyl alcohol indicated that a reaction has occurred toward CH_2OH. Together with the presence of a strong C=O absorption band appearing at 1778 cm^{-1}, therefore, this indicated that salicyl maleic acid has been obtained in this experiment.

It has been reported previously (Jumina, 2000 and 2001) that ester form of C-9154 antibiotic derivatives could be prepared through esterification of the related acid using concentrated sulfuric acid as the catalyst. Referring to this report, then the acid form of C-9154 antibiotic derivative obtained was esterified using absolute ethanol in the presence of concentrated sulfuric acid. The reaction was conducted at reflux for quite a long time (4 hours) as examination using thin layer chromatography (TLC) indicated that the reaction has not gone to completion after being heated for 2.5 hours.

Identification of the product using proton NMR spectrum led to a conclusion that the product was not the right compound. This arose as the spectrum displayed only a singlet of alkenyl protons at 6.2 ppm instead of the expected two singlets even though the existence of CH_2CH_3 group is clearly indicated in the spectrum. In addition, the data indicated that the expected esterification was also followed by transesterification which replaced the *o*-hydroxyphenoxy group by ethoxy group leading to the formation of

diethyl maleat. Such transesterification is possible as *o*-hydroxybenzyl group is sufficiently bulky so that replacement by smaller ethyl group would afford to a thermodinamically more stable product.

Due to the above difficulty, then the esterification of salicyl maleic acid with ethanol was performed using benzenesulfonic acid which is weaker than sulfuric acid. The experiment was conducted at reflux for 3.5 hours to give yellow oil of the desired ethyl salicyl fumarate in 83 % yield. The formation of ethyl salicyl fumarate as the final product rather than ethyl salicyl maleate was concluded on the basis previous findings, which showed that maleic fragment (cis) could easily be isomerized to fumaric fragment (trans) through heating at 75-100^{o}C for 1-2 hours (Vogel, 1968). It was supposed that the heating (78^{o}C) undertaken in this experiment that was sufficiently long (3.5 hours) could simultaneously converted ethyl salicyl maleate to ethyl salicyl fumarate.

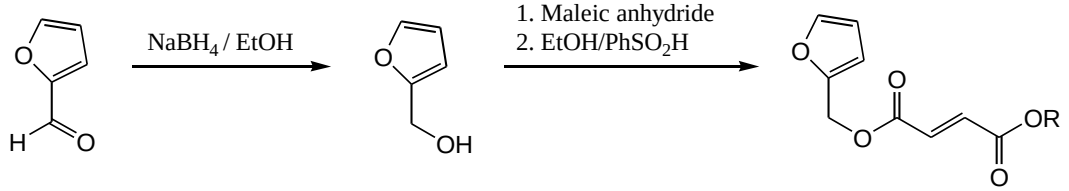
Identification of the product using IR spectrum still showed the existence of OH group appearing at 3392 cm^{-1}. However, the peak is less intense and not as wide as that of salicyl maleic acid, which perhaps indicates that esterification has already occurred toward the COOH group. Another indication for the occurrence of eterification is the enhancement of CH_3 bending frequency appearing at 1247 cm^{-1} in comparison to that of salicyl maleic acid. Thus, it could be shown in here that the esterification has occurred without cleaving the *o*-hydroxybenzyl group.

The proton NMR spectrum of the resulted ethyl salicyl fumarate showed a triplet at 1.2 ppm (3 H) and a quartet at 4.2 ppm (2 H) indicating the existence of CH_2CH_3 functionality. The maleic CH=CH protons resonate as two singlets at 6.15 and 6.30 ppm, whereas the phenyl and methylene protons respectively appear at 6.4-7.2 ppm and 3.8 ppm. Although the spectrum did not exhibit a proper integration, the spectrum could clearly proved that the outcome of the reaction is the expected ethyl salicyl fumarate.

Synthesis of ethyl furfuryl fumarate

As done for ethyl salicyl fumarate, the preparation of ethyl furfuryl fumarate from furfural was conducted in 3 steps i.e. (1) reduction of furfural, (2) condensation of furfuryl alcohol with maleic anhydride, and (3) esterification of furfuryl maleic acid (Scheme 2). This method of preparation is different from the that reported by Sumiratinah (2000) who prepared C-9154 antibiotic derivatives through the condensation of furan-2-acrylate with maleamide.

The reduction of furfural was conducted using sodium borohydride in refluxing ethanol for 2 hours. Heating at reflux (78^{o}C) was required as most of starting materials remained intact when the reaction was performed at room temperature. Furfuryl alcohol was obtained in 53 % yield and was found as brownish-yellow oil.



furfural *furfuryl alcohol* *furfuryl maleic acid (R = H; **8**)*
*ethyl furfuryl fumarate (R = Et; **9**)*

Scheme 2

The IR spectrum of the synthesized furfuryl alcohol showed the disappearance of C=O stretching frequency appearing at 1674 cm^{-1} in the IR spectrum of furfural. Instead, the spectrum demonstrated the existence of an intense broad band of OH group appearing at 3336 cm^{-1}. These phenomena, therefore, proved that the reduction of the furfural C=O group has occurred.

Further analysis of the synthesized furfuryl alcohol was conducted using GC-MS spectrometer. The chromatogram showed that the compound was sufficiently pure. The only peak appearing in the chromatogram gave molecular ion at *m/z* = 98 on its mass spectrum. This *m/z* value is exactly the same as the

value of molecular weight of furfuryl alcohol. Other fragments appearing at *m/z* = 81 and *m/z* = 69 were supposed to be generated respectively through the fragmentation of that molecular ion by loosing OH and CHO groups. Thus, there is no doubt that the reduction of furfural C=O group has been achieved in this experiment.

The condensation of furfuryl alcohol and maleic anhydride was conducted in benzene at 50-60^{o}C for 2 hours to give 72 % yield of furfuryl maleic acid which was found as dark-brown oil. The product was identified using IR spectrum which showed the existence of OH and C=O stretching frequencies of the COOH group respectively at 3500 and 1740 cm^{-1}. In addition, the existence of maleic fragment was also indicated by the presence of a medium absorption band at 1635 cm^{-1} which corresponded to the stretching frequency of C=C bond.

As a final step, the resulted furfuryl maleic acid was treated with excess ethanol in the presence of catalytic amount of benzenesulfonic acid at reflux for 1.5 hours to afford the expected ethyl furfuryl fumarate in 38 % yield which was found as dark-brown oil. This relatively low yield perhaps was attributed by the instability of the furan ring especially under acidic and thermal conditions. Indeed, it was discovered that only polymeric material was obtained when the esterification was conducted using concentrated sulfuric acid as the catalyst. Likewise, polymeric material was the major product when the reaction was conducted at reflux for 3.5 hours.

Identification of the synthesized ethyl furfuryl fumarate was performed using IR spectrometer. The IR spectrum clearly showed the disappearance of OH stretching frequency appearing at 3500 cm^{-1} in the IR spectrum of furfuryl maleic acid. This proved that a reaction has occurred to the hydroxyl moiety of the COOH group. In addition, the IR spectrum exhibited a strong absorption band of C=O stretching frequency at 1724 cm^{-1}. Whereas CH and C-O stretching frequencies respectively appeared at 2931-2981 and 1161 cm^{-1}, C=C stretching frequency appeared at 1617 cm^{-1}. Although this product has not been characterized fully, the IR spectrum obtained should provide meaningful evidence for the formation of ethyl furfuryl fumarate.

Antimicrobe activity of the synthesized C-9154 antibiotic derivatives

Antimicrobe activity evaluation towards the synthesized C-9154 antibiotic derivatives was conducted using *Staphyllococcus aureus* and *Eschericia coli* respectively as the representative of Gram positive and Gram negative bacteria. Aqueous methanol was used as the control, and antimicrobe activity was determined through measurement of minimum inhibition concentration (MIC) of the compound towards the growth of the bacteria. The MIC values obtained for salicyl maleic acid **(6)**, ethyl salicyl fumarate **(7)**, furfuryl maleic acid **(8)**, and ethyl furfuryl fumarate **(9)** towards *Staphyllococcus aureus* and *Eschericia coli* are presented in Table 1.

Table 1 MIC values of C-9154 antibiotic derivatives

Compound	**MIC towards *Staphyllococcus aureus* (μg/mL)**	**MIC towards *Eschericia coli* (μg/mL)**
Salicyl maleic acid **(6)**	500	2000
Ethyl salicyl fumarate **(7)**	100	4000
Furfuryl maleic acid **(8)**	150	150
Ethyl furfuryl maleic **(9)**	100	100

As shown in Table 1, compound **6**, **7**, **8**, and **9** are all active against *Staphyllococcus aureus* and *Eschericia coli* even though compound **6** and **7** only show weak antimicrobe activity against *Eschericia coli.* Accordingly, it could be concluded to some extent that the synthesized C-9154 antibiotic derivatives are more effective against the Gram positive bacteria. The data also show that in general the ethyl ester forms of the C-9154 antibiotic derivatives obtained are more active than the carboxylic acid analogues. Thus, this finding is consistent with the discovery previously reported (Jumina, 2001).

Compared to compound **2**, **3**, **4**, and **5**, which showed MIC values towards *Staphyllococcus aureus* and *Eschericia coli* in the range of 400-2500 μg/mL, it can be pointed out that in general compound **6**, **7**, **8**,

and **9** exhibit stronger antimicrobe activity. Thus, the attachment of phenolic hydroxyl group, which increases the polarity of the compound, could also enhance the antimicrobe activity. Prominent enhancement of activity compared to those of compound **2**, **3**, **4**, and **5** is even found for compound **8** and **9** showing antimicrobe activity of 100-150 μg/mL which are already comparable to the MIC values (25-150 μg/mL) of some common antibiotics such as phenycillin, chemicitine, or amoxyline (Burger, 1960 and Martin, 1982). Therefore, it can also be concluded that the existence of furan ring instead of benzene ring in the structure of C-9154 antibiotic derivatives could significantly enhance their antimicrobe activity. Unfortunately, due to its instability, compound **9** was rather difficult to synthesize, and the yield obtained in this experiment was only 38 %.

CONCLUSIONS

Ethyl salicyl fumarate **(7)** could be prepared in good yield from methyl salicylate through reduction followed by condensation with maleic anhydride and esterification with ethanol using benzenesulfonic acid as the catalyst. The similar procedure could also be applied for the synthesis of ethyl furfuryl fumarate **(9)** even though the yield was rather unsatisfacty.

Furfuryl maleic acid **(8)** and ethyl furfuryl fumarate **(9)** are sufficiently effective to inhibit the growth of *Staphyllococcus aureus* and *Eschericia coli*. In contrast, salicyl maleic acid **(6)** is not significantly active towards *Staphyllococcus aureus* and *Eschericia coli*. In the case of ethyl salicyl fumarate **(7)**, this compound is sufficiently active against *Staphyllococcus aureus*, but is not sufficiently active against *Eschericia coli*.

The existence of phenolic hydroxyl group and especially furan ring in the structure of C-9154 antibiotic derivatives could significantly enhance their antimicrobial activity.

ACKNOWLEDGEMENT

The authors deeply thank to INDONESIA TORAY SCIENCE AND TECHNOLOGY FOUNDATION for its financial support for the implementation of most of the research activities.

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