Journal of Applied Pharmaceutical Science Vol. 4 (01), pp. 001-008, January, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.40101
ISSN 2231-3354

Synthesis of Dihydroartemisinin using Ni/TiO_2 catalyst Prepared by Sol Gel Method

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ARTICLE INFO

Article history:
Received on: 19/11/2013
Revised on: 22/12/2013
Accepted on: 24/01/2014
Available online: 30/01/2014

Key words:
Dihydroartemisinin, Synthesis, Hydrogenation, Ni/TiO_2 catalyst, sol gel.

ABSTRACT

Artemisinin is a δ-sesquiterpene lactone that incorporates an endoperoxide moiety. This compound was isolated as the active compound of *Artemisia annua* L. Dihydroartemisinin is the simplest semisynthetic derivative of artemisinin and is more potent than artemisinin. Combined with piperaquine, curently this compound is the drug of choice to treat malaria. The objective of this research is to modify the structure of artemisinin into dihydroartemisinin. A new way to modify the structure of artemisinin into dihydroartemisinin, had been successfully conducted using hydrogenation process with Ni/TiO_2 catalyst. The yield of the reaction was 16.58%. LC-MS analysis showed that the compound had mainly a peak with retention time t_R 2.2 minutes and mass spectrum showed that the molecular weight of the compound was 284.29 which was the molecular formula of dihydroartemisinin, $C_{15}H_{24}O_5$. The IR spectrum showed that there was a spectrum from C-O in a wave number of 1034.14 cm^{-1}. Hydrogenation reaction did not destroy the endoperoxide group. This was proven by the existing of C-O-O-C in a frequency of 1091.71; 875.68; 844.82 cm^{-1}. ^{1}H- and ^{13}C-NMR data and comparison to the authentic sample, showed that the compound was a racemic mixture of α/β dihydroartemisinin. The dihydroartemisinin resulted from this process was also proven for its antimalarial activity, in vitro assay using cultured *Plasmodium falciparum* clone 3D7 with its IC_{50}= $2x10^{-7}$M.

INTRODUCTION

Artemisinin was originally developed in China in 1972 by the Institute of Chinese Medicine, a bioactive component of the plant *Artemisia annua* L. (sweet wormwood). In 1979 the structure of artemisinin has been successfully elucidated with the empirical formula $C_{15}H_{22}O_5$ and systematic name is [3R-(3α, 5aβ, 6β, 8aβ, 9α, 12β, 12aR)]-oktahidro-3,6,9-trimethyl-3, 12-epoxy-12H-pirano [4,3-j] -1,2-bensodioxepin-10-(3H)-one (Liao, 2009; Wright et al. 2010). In China in 1979, approximately 2,099 patients were infected with *P. vivax* and *P. falciparum* and treated with artemisinin. The results were very good and without any side effects. WHO has recommended the use of combination anti-malaria drugs to cope with chloroquine resistant malaria, especially falciparum malaria (WHO, 2005). Artemisinin is one of the main components in the combination, effective for cerebral malaria or tropical malaria, and has proven that the patient's body temperature can be normalized within 72 hours whereas asexual parasites can be eliminated within 72 hours. Even in Vietnam, artemisinin has been tried is given to children aged 1 to 15 years who are selectively chosen in order not infected with the parasite *P. falciparium* and it proved to work well. Various extraction of artemisinin from *Artemisia annua* was reported (Christen, 2001). Previous various studies indicated that artemisinin had the ability to break the chain of the parasite's life cycle in red blood cells, and there has been no case reports of artemisinin-resistant malaria. But the clinical application of artemisinin as a therapeutic agent is limited by several factors. Artemisinin has a relapse rate within 8 weeks (rate of recrudescence) high, its oral activity lower in the desired dose, the half-life in plasma is low and limited solubility in both water and oil (Lin and Miller, 1995).

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In addition, artemisinin can also decompose the proton-containing solvent. Therefore, efforts to address the issue of therapeutic and pharmaceutical, among others, through the modification of the chemical structure of a compound artemisinin analogs and is expected to provide several advantages and benefits compared to the parent compound. One of the water-soluble derivatives of artemisinin is dihydroartemisinin, while the oil is soluble ether group that artemether and artether (Klayman, 1985). Dihydroartemisinin an artemisinin derivative are soluble in water. Modification of artemisinin into dihydroartemisinin done through the reduction. On artemisinin bound carbonyl group (C = O), while the bound dihydroartemisinin hydroxy group (C-OH). Conversion into hydroxy carbonyl group is known as the reduction. Reduction can take place with the aid of a reducing agent. The most commonly used reductant is $LiAlH_4$ and $NaBH_4$. Several previous studies on the synthesis dihydroartemisinin using $NaBH_4$ catalyst at the reduction process, was reported that the $NaBH_4$ reductor reducted lactone carbonyl group without breaking the peroxide bond (Gedre *et al.,* 1997; El-Feraly *et al.,* 1990; Vishwakarma, 1990). In contrast to studies that have been done before, in this study dihydroartemisinin be synthesized by reducing with solid catalysts that are expected to obtain artemisinin derivative with a yield greater, purer and easier in the process of separation. To obtain compounds with high purity required a kind of catalyst that has the good physical properties, that is nano-sized mesoporous metal catalyst with is able to hydrogenised. This study uses the stages of preparation of support solid catalysts TiO_2 were impregnated with Ni by sol-gel method. Support can affect the adsorption and activity/selectivity of the hydrogenation of the carbonyl group by nickel. Hydrogenation of artemisin into take place dihydroartemisinin using nickel-metal catalysts that have reduction properties. In addition, metals such as Rh, Ru, Pt and Pd are also reported to have a high activity but is economically less profitable because the price is very expensive. The process that occurs is dependent on the structure of artemisinin carbonyl group to the metal surface so that its stability disturbed. Hydrogen will be entered into the structure and formed dihydroartemisinin. From literature searches to be known that the synthesis of artemisinin derivatives in general using $NaBH_4$ or $LiAlH_4$ which is a liquid catalyst process in a liquid-liquid separation. The use of solid catalysts Pd / C as a reducing agent in the synthesis of artemisinin derivatives has also been studied to be but still shows some weaknesses such as a very long reaction time (20 hours), the outbreak of the bridge tends to eliminate peroxide antimalaria activity. Therefore, in this study used a solid catalyst Ni/TiO_2 be expected to synthesize artemisinin derivatives without damaging peroxide groups that still have antimalaria activity and easier in the process of separation (Zhang *et al.,* 2010; Singh and Tiwari, 2002; Lee, and Oh, 2002;. Galal *et al.,* 2009).

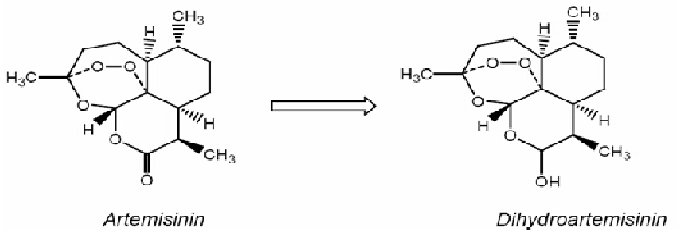
RESULTS AND DISCUSSION

Crystallization behaviors of the gels were investigated with X-ray diffractometer (PhilipXpert XRD). A totalof about 2 g of powdered catalyst was placed on the sample plate, and then was analyzed. 2θ angle measured was 10-80^o with a measurement time of 23 minutes, CuKα source, the target Cu and λ = 1.54 nm. Peaks of sample were compared with computer data base to determine the type of crystal. X-ray diffraction analysis was conducted to determine the crystal structure and determine the diffraction patterns of the catalyst samples. Crystallite size was calculated using Debye-Scherrer equation. Figure 1, shows the peaks of Ni/TiO_2 catalyst after drying, calcination and reduction. The peaks of Ni/TiO_2 after reduction were identified the characteristics of the Ni peak, at 2θ = 25.34 area. From the results of calculations using Debye-Scherrer equation, crystallite size of 8.4 nm was obtained. This suggests that the catalyst after calcination and reduction treatments seem that the catalyst has a high intensity Ni/TiO_2 at an angle 25.34. The form of pure TiO_2 suport crystal diffraction pattern of anatase $TiO_2$was in accordance with the ASTM 04-0477 because it was found to peaks at 2θ = 25.34^o district (100%), 47.88^o (24.9%) and 54.14^o (24.4%). Calcination conduct gave rise to the characteristic peak of NiO at 2θ = 37.34^o (26.7%), 43.32^o (24.4%) and 62.86^o (28.9%). On the identification of the characteristic peak Ni after reduction found at 2θ = 43.32^o (24.4%) and 54.14^o (24.4%) corresponds to the diffraction pattern of Ni in ASTM 04-0850. From these data it can be estimated that the reduction process do not run properly in changing the nickel oxide into nickel metal, even at an angle of 43.32 and 54.14^o of Ni formation was visible reduction of revenue, but the intensity is still low. This may be because the percentage of Ni on TiO_2 was still too big. By using this nano-catalyst, selectivity and high efficiency can be improved because it can work at a lower energy level. Where, catalytic activity was determined by the size of the pores of the catalyst. Specific surface area, S_{BET} calculated using the BET equation, while the pore size distribution curves obtained through analysis BJH adsorption using the method of calculation. From the analysis found that the TiO_2 catalysts have not been impregnated nickel specific surface area of 4.71 m^2/g and a total pore volume of 0.0019 cm^3/g. Ni impregnation treatment and reduction process, increase both of the specific surface area, and the total pore volume to be 47.36 m^2/g and 0.102 cm^3/g respectively. Specific surface area catalysts undergo changes after impregnation process, calcination and reduction, as long as the process is the addition of a solvent or temperature treatment that will lead to changes in species or solvent adsorption of nickel and nickel crystal size.

Dihydroartemisinin compound was synthesis via reduction of artemisinin by Ni/TiO_2 catalyst in methanol for 5 hours at 100 ° C and a pressure of 5 bar. Activities that occur during the hydrogenation reaction can be explained as follows (Figure 2.). It was originally a metal catalyst which will separate hydrogen gas into the atoms and stabilize the hydrogen atoms in the radical form, thus forming H_2 and H• (radical). Hydrogen atoms which have been activated are highly reactive and will try to get a new stability with full s orbital (with 2 electrons). Meanwhile, the simultaneous double bond in artemisinin also have been broken down by metal catalysts. After that, the hydrogen

atom will attract electrons from other nearby chemicals (which is in the case of artemisinin hydrogenation, hydrogen will break the bonds between the carbon atoms 10^{th} of artemisinin with oxygen and replaces the position of the two bonds with new bonds with hydrogen atoms) .

Reaction occurred in the process of modification of artemisinin into dihydroartemisinin by Ni/TiO_2 catalyst via hydrogenation process was as follows:



At the end of the hydrogenation, the result was a yellowish solution. Evaporation of the solvent with rotary evaporator produces yellowish-white oily solid weighing 99.7 mg (99%). Oily solids were still a mixture of the target compound and several other compounds. The results of purification by column chromatography using hexane and ethyl acetate solvent obtained target compound in the form of white crystals that dissolve completely in methanol and chloroform of 18.3 mg (16.58%) with a melting point of 151-153 ^{o}C.

TLC analysis of the solvent toluene-ethyl acetate-formic acid (7:3:1) gave 1 spot stain. Staining results with p-anisaldehide reagent gave yellowish pink spot with R_f0.44. Target molecule was more polar than the artemisinin. TLC of the data indicated that for the pure target compound chromatogram appeared only one spot. To determine the purity, these compounds need to be further analyzed with the other chromatographic methods. Synthesized compounds were analyzed by liquid chromatography-mass spectrometry (LC-MS), Infrared spectrophotometer (FT-IR) and ^{1}H- and ^{13}C-NMR spectrometer. FT-IR spectra were used to determine the type of functional groups present in the molecule compounds. ^{1}H- and ^{13}C-NMR spectra were used to determine the number and position of the proton and carbon compounds synthesized. While the mass spectrum used to determine molecular weight compounds. Chromatograms obtained were compared with chromatograms of artemisinin the starting material.

Initial testing was conducted by using Thin Layer Chromatography (TLC) as a first step to test the separation of thin layer chromatography. The results of synthesis was analyzed by TLC using silica gel F_{254} as stationary phase and the mobile phase (eluent) toluene-ethyl acetate-formic acid resulted six spots. The spots were seen clearly and sprayed with anisaldehide reagent. These spots showed yellowish pink color. The R_f value changes were compared to the starting compound reaction products, showed that the synthesis of target compound reaction has taken place. The synthesis results still consisted of the target compound and several other compounds. Therefore the results were separated by column chromatography with a diameter of 0.5 cm and length 30 cm column. The base of the column that was washed away dried and given a bit of cotton, the mobile phase (eluent) was passed into the column to wet the cotton. The prepared 300 mg silica gel (E. Merck 7733) was the stationary phase. Silica gel was taken piece meal and diluted with *n*-hexane in a small glass beaker and stirred until homogeneous and there are no air bubbles. Silica gel was added slowly using a funnel into the column until they run out. Synthesized sample was mixed with silica gel, a little diluted with *n*-hexane and then inserted into the column. Inserted eluent (mobile phase) starts from n-hexane 100% to hexane ratio with ethyl acetate were 1%, 2%, 5%, 10%, 15%, 20%, 25% and 50%. Solution coming out of the column collected in each vial 10 mL. Then solution in each vial was tested by TLC. Compounds containing a single stain with R_f 0.44 were collected, since this compound is similar with compound targets that showed the same R_f with dihydroartemisinin standard. This compound was then concentrated by evaporating the solvent. Target compound obtained from the purification produced fine white needle crystals which weighed 18.3 mg (16:58%).

The results obtained in this reaction is not high enough when compared with the general reduction products using $NaBH_4$ or using Pd catalyst. This is due to not optimal reaction conditions used. The success of a reduction is determined by the type of reductant addition, other factors such as the solvent, the contact time between the substrate and the reducing agent, reaction temperature, stirring speed and duration that will also affect the yield of the results. Reduction of artemisinin by $NaBH_4$ produced dihydroartemisinin (DHA), and yielded a new stereochemically labile centre at C-10, which, in turn, provided two inter-converting lactol hemiacetal epimers (namely α and β), whose rate of inter-conversion depends on buffer, pH, and solvent polarity (D'Acquarica, 2010).

Target compound analysis by LC-MS giving a peak with a retention time (t_R) 2.2 min. After separation by liquid chromatography, electrospray ionization mass spectrometry (ESI-MS) was performed to determine the ion-ion and fragments in the molecule. When the ESI-MS use positive ion mode, molecules formatted with addition of ions such as $[M + Na]^+$, $[M + H]^+$, $[M + H]^+$, $[2M + Na]^+$, [2M + K]+, $[M + NH_4]^+$ and other positive ions [15].LC-MS measurements yielded the target compound that has the molecular formula $C_{15}H_{24}O_5$ and molecular weight 284 g/mol. It is shown that this compound gave the molecular ion peak at 285.28 $[M + 1]^+$, but the peak is not significant and is not a dominant peak. Dominant molecular ion peak at 100% intensity appear as $[2M + Na]^+$ that is at m/z 590.85 $[2M + 23]^+$, as shown in the figure 3. As the result of targeted compound screening mass spectrum, molecular ion peak appeared at m/z 284.29, but very low and accompanied the emergence of $[M + H]^+$ molecular ion at 285.28. It is well suited to study ever done on the analysis of artemisinin in *Artemisia annua* L. using LC-MS, where the dominant molecular ion peak is the $[M + Na]^+$ and $[M + H]^+$and the ion peak does not appear as a significant molecular since the ion peak is not as dominant in determining the type of positive ions (Wang *et al.,* 2005). In the IR spectrum of emerging

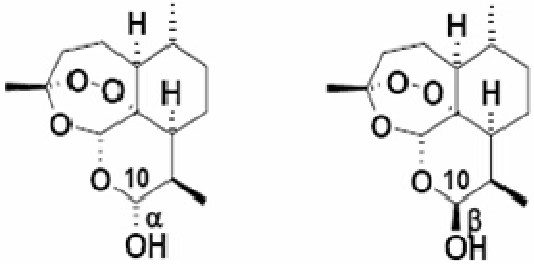
artemisinin absorption frequency 1735.93 cm^{-1} which is the C=O stretching vibration of C=O stretching vibration of cyclic ketones and cyclic ester (lactone) and the target compound was no longer on the uptake at area with that frequency. In this study, the process of change of artemisinin to the target compounds via hydrogenation is characterized by the emergence of regional uptake at 3371.57 cm^{-1} frequency which is the stretching vibration of the-OH group, Figure 4. This is supported by the appearance of absorption bands of C-O stretching vibration at a frequency of 1034.14 cm^{-1}. The-OH stretching vibration appeared at 3390 cm^{-1}dihidroartemisinin (El-Feraly et al, 1990). Hydrogenation does not destroy the existence of the endoperoxide group. This is proven by the presence of C-O-O-C stretching vibration absorption at frequency 1091.71; 875.68; and 844.82 cm^{-1}. These data indicate that the target compounds containing hydroxyl group (-OH) and peroxide (C-O-O-C).

To equip the spectroscopic measurement data, measurement of NMR spectra was carried out. ^{13}C NMR was used to determine the number of carbon atoms and determine the types of carbon atoms in the compound. Carbon nuclear magnetic resonance spectrum of target compounds containing 15 carbon atoms, which consists of 3 methyl group (CH_3), 4 methylene group (CH_2), 5 metin group (CH), and 3 C atoms quartener. On the spectra indicated the presence of chemical shift in the value of C-10 is on artemisinin δ 172.24 ppm while the target compound δ 96.60 ppm. This indicated a change of carbonyl groups into hydroxyl groups according to the value of the predictive value of the chemical shift of dihydroartemisinin. In the ^{13}C NMR spectra can also be seen that there are three methyl groups at the 3-Me, 6-Me and 9-Me with a shear value of chemistry in 13:37, 20:56 and 26.26 ppm, respectively. ^{1}H NMR data is to see the position of carbon atoms with or without a proton. Besides that it will be known other atoms related to the proton. ^{1}H NMR spectral analysis of the data indicated a hydroxyl group (-OH) in the chemical shift of δ 2.77 ppm (singlet, 1H). In addition, the spectrum showed the presence of a proton from the-OH group at C-10 which consists of 10α and 10β, respectively with the chemical shift of 4.75 ppm (triplet, 1H) and 5.60 ppm (singlet, 1H), and this value is not found at the artemisinin compounds. This also reinforces a change in the C-10 into hydroxyl. Other proton signals showed the presence of three methyl group that is the chemical shift at 1.43 ppm (singlet, 3-Me) namely methyl which had not a neighbor carbon bonded to hydrogen. The chemical shift at 0.96 ppm in the form of a doublet (d) is a methyl group with carbon neighbors hydrogen atoms bonded to one that shows the 6-Me and 9-Me with the integration of 3H, respectively. The signal indicates a methyl group at atom C-3, C-6 and C-9 are reinforced by data $^{13}CNMR$.

In addition, the ^{1}H NMR spectrum (Figure 5) showed a methylene group at atom C-4, C-5, C-7, C-8, which gives multiplet signal. In the C-4 atom consists of 4α and 4β, 4α where the chemical shift of 1.82 ppm (dd, 1 H) and the chemical shift of 1.76 ppm 4β (dd, 1 H) 1 atom bound with hydrogen and with the influence of methyl groups on atom C-3 will also take place that give signal coupling doublet. On atom C-5 signaling in chemical shift 1.70 ppm and 1.47 ppm (multiplet, 2H) which is a two-proton chemical shift of the CH_2 groups attached to the carbon with the neighboring hydrogen atom 5. At δ = 1.23 and 1.64 ppm (multiplet, 2H) indicated a two-proton chemical shift of the-CH2, at δ = 1.67 and 1.28 ppm (multiplet, 2H) indicated a two-proton chemical shift of the-CH2. At δ = 1:24 ppm (multiplet, 1H) indicated the chemical shift of the proton-CH group of atoms C-5a, δ = 1.33 ppm (multiplet, 1H) indicated a shift of the proton-CH group of atoms C-6, δ = 1.49 ppm (multiplet, 1H) indicated a shift of the proton-CH group of atoms C-8a, δ = 2.30 ppm (quartet, 1H) indicated a shift of the proton-CH group of the C-9 atom bound to three neighboring carbon with the hydrogen atom, δ = 5.29 ppm (singlet, 1H) indicated shift of the proton-CH group of atoms C-12 that do not have neighbors bonded carbon and hydrogen which appears downfield because adjacent to the oxygen atom. While there is a quaternary C at C-3, C-12a and C-10. Based on the literature study note that dihydroartemisinin generated from artemisinin synthesis usually results in a racemic mixture comprising the α and β forms. This is indicated by the signal at 4.78 ppm in the form of a triplet (t) by integration of the 1H by partner 5:38 shaped ppm 1H singlet with integration is dihydroartemisinin in the form of α and β forms are characterized by the presence of a signal at 5.60 ppm in the form of singlet (s) with the integration of 1H.

Dihydroartemisinin was a mixture of two epimers the 10α-hydroxyl and the 10β-hydroxyl. Epimer configurations differ only in the C-10 atom is the center of lactol epimer. Comparison between 10α and 10β epimers in solution is 1:1 chloroform, acetone 2:1, and 3:1 dimethyl sulphoxide were determined by NMR spectroscopy determination.

Dihydroartemisinin can be converted into artemether and artesunate. Derived from β-artemether and artesunate isomers of α-isomer. OH group on the β-isomer has greater steric hindrance thus do not allowing for succinic anhydride to attack the group. Whereas the-OH group on the α-isomer is in the form of equatorial and less interaction with neighboring groups, so succinic anhydride can be easily attacked. α-isomer has a very high stability than the β-isomer [12].



DihydroartemisininEpimers

The value of changed R_f and supported by spectroscopic data indicated that the synthesis reaction has taken place and the compound artemisinin compounds synthesized in the form of a dihydroartemisinin racemic mixture.

ά/β Dihydroartemisinin: White crystline powder, mp 141-143 ^{o}C, LCMS *m/z*285.28 $[M + 1]^{+}$,590.85 $[2M + 23]^{+}$,^{1}H-NMR d 1.82; 1.76 (2H)(H-4), 1.70; 1.47 (2H,m)(H-5), 1.24 (1H,m)(H-5a), 1.33 (1H,m)(H-6), 1.64;1.23 (2H,m)(H-7), 1.67;1.28 (2H,m)(H-8, 1.49 (1H,m)(H-8a), 2.30 (1H)(H-9), 5.60 (1H, d, H-10β); 4.75 (1H, s)(H-10 β), 5.29 (s,1H)(H-12), 1.43 (3H, s)(3-Me), 0.96 (3H)(6-Me), 0.96 (3H)(9-Me). 13C-NMR d 104.31 (C-3). 36.56 (C-4), 24.90 (C-5), 51.72 (C-5a), 37.67 (C-6), 34.89 (C-7). 30.96 (C-8), 44.51 (C-8a), 35.00 (C-9), 96.60 (C-10), 87.96 (C-12), 81.31 (C-12a), 26.26 (Me C-3), 20.56 (Me C-6), 13.37 (Me C-9). This data is comparable to previous published report [14, 16].

The synthetic result in concentrations of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} M was evaluated for its antimalaria activity in *vitro assay*, using cultured of *Plasmodium falciparum* clone 3D7 and chloroquine as the positive control. The racemic mixture of dihydroartemisinin showed antimalarial activity and the IC_{50} value was $2x10^{-7}$ M and comparable to cloroquine IC_{50} vale was $3x10^{-9}$ M.

Experimental Section

Materials used for the preparation of the catalyst is titanium iso-propoxide (Fluka Chemical), nickel nitrate (E. Merck), sulfuric acid (E. Merck), nitric acid (E. Merck), n-propanol (E.Merck) and CTAB, centyltrimethyl-ammonium-bromide (E. Merck). For the synthesis reaction and purification using artemisinin isolated from *Artemisia annua* L. which is obtained from PT. Sinkona Indonesia Lestari. Another materials for experiment were : HP hydrogen gas, methanol pa (E. Merck), ethyl acetate, n-hexane, toluene, formic acid, chloroform, reagent *p*-anisaldehide as TLC stain apparition, DMSO (dimethyl sulphoxide), Silica gel 60 GF 254 (70-230 mesh, Merck) and TLC plates (E. Merck 05554).

The results were analyzed by XRD, infrared spectrophotometer (Perkin Elmer 16 PC, FTIR, Shimadzu Prestige-21), nuclear magnetic resonance (500 MHz, JEOL), Liquid Chromatography-Mass Spectrometry Biored Mariner (70 ev), melting point determination tool Fisher Scientific 903N series 0056. LAF (Laminar air flow Forma Scientific).

Preparation of Ni/TiO_2 catalyst by sol-gel method

Wet gels of TiO_2 were prepared by hydrolysis of Titanium Isopropoxidein n-propanol solution with acid catalyst. Firstly, 14.45 n-propanol was stirred at 0 ° C for a few minutes then added Titanium IsoPropoxide 9.25 ml. The solution then added HNO_3 until mixture reaches pH 3. Mixture at reflux for 5 hours at 70 ° C and then added water until the gel is formed. Wet gel formed was aging and immersed surfactants in n-propanol at room temperature for 24 hours. Furthermore the solvent was decanted and dried in the oven overnight at 70 ° C (surfactant modified gel). The gel was then calcinted at 500^{o}C for 8 hours using a furnace. Subsequently impregnated with $Ni(NO_3)_2$ with equimolar ratio and stirred at 50 ° C until the water evaporates and the slurry was formed. Dry gel formed was dried for 3 hours at a temperature of 70^{o}C. Gel was then calcined at 500^{o}C for 8 hours using a furnace. Catalytic reduction process was then performed at a temperature of 300 ^{o}C for 4 hours. Dihidroartemisinin synthesis was performed as follows: A 100 mg (0.35 mmol) of pure artemisinin (Aldrich) carefully weighed and then added 30 ml of methanol and 10 mg of catalyst Ni/TiO_2, inserted in the reactor and heated for 3 hours at a constant temperature of 100 ^{o}C. After that, the flow of hydrogen gas at the same temperature for 5 h at a constant pressure of 5 bar. Product was then filtered to be separated from the catalyst and dried. The filtrate was then concentrated using a rotary evaporator. Concentrated extracts were then purified by column chromatography and then tested by thin layer chromatography and melting point and compared to the authentic racemic sample (Aldrich).

Antimalarial evaluation

The antimalarial evaluation was conducted using an established published methods (Rocha e Silva *et al.,* 2011; Fidock *et al.,* 2004). Continuous culture of Plasmodium falciparum. Strains used in this study were the antimalarial drug-susceptible3D7 clone obtained from the Eyckman Instintute, Jakarta. Parasites were maintained in continuous culture using the method of Trager and Jensen at 5% hematocrit using type A+ human erythrocytes in RPMI 1640 medium supplemented with 2 mM L-glutamine, 25 mM HEPES, 40 μg/mL gentamycin, 10% A+ humanplasma, 25 mM $NaHCO_3$ (Trager and Jensen, 1976). Cultures were maintained under an environment of 5% O_2, 5% CO_2 and 90% N_2 and incubated at 37 °C. When cultures attained a parasitemia of 4–5% they were synchronized with 5% sorbitol. Detailed established procedure can be seen in previous report (Rocha e Silva *et al.,* 2011). The chloroquine solution at 10^{-5}-10^{-9} M concentrations were used as the positive control.

CONCLUSION

New ways to modify the structure of artemisinin derivatives dihydroartemisinin have successfully been done through catalytic hydrogenation reactions using Ni/TiO_2. Synthesis of dihydroartemisininin needle shaped crystals with yield 16:58% and a melting point of 141-143^{o}C, was obtained. TLC analysis with silica gel 60 F_{254} plates using toluene eluent: ethyl acetate: formic acid showed one spot with R_f 0.44. LC-MS analysis showed a single peak with t_R 2.2 min and the molecular weight 284.29 and molecular formula $C_{15}H_{24}O_5$of dihydroartemisinin. IR spectrum of dihydroartemisinin showed a hydroxyl group at 3371.57 cm^{-1} frequency supported by the emergence of absorption band of C-O stretching vibration at a frequency of 1034.14 cm^{-1}. Hydrogenation reaction does not destroy the existence of the endoperoxide group. This is proven by the presence of C-O-O-C stretching vibration absorption at frequency 1091.71; 875.68; 844.82 cm^{-1}. From the NMR data prove that the compound is a racemic mixture of α / β dihydroartemisinin. The racemic mixture of dihydroartemisinin showed antimalarial cultured *Plasmodium falciparum* clone 3D7 with its IC_{50} of $2x10^{-7}$ M.

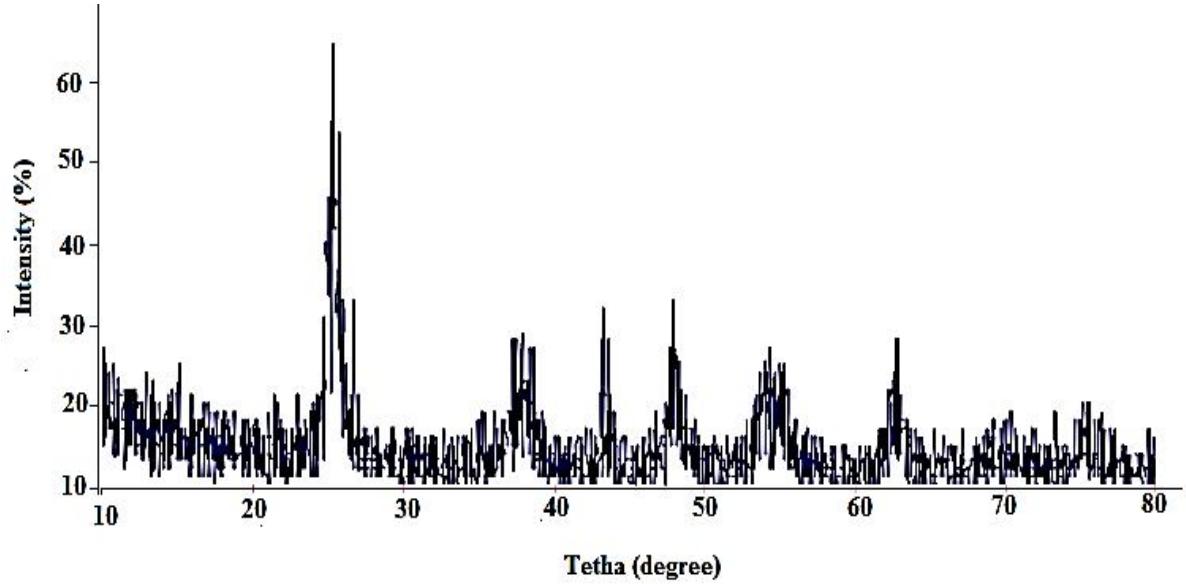


Fig. 1: XRD pattern of Ni/TiO_2catalyst.

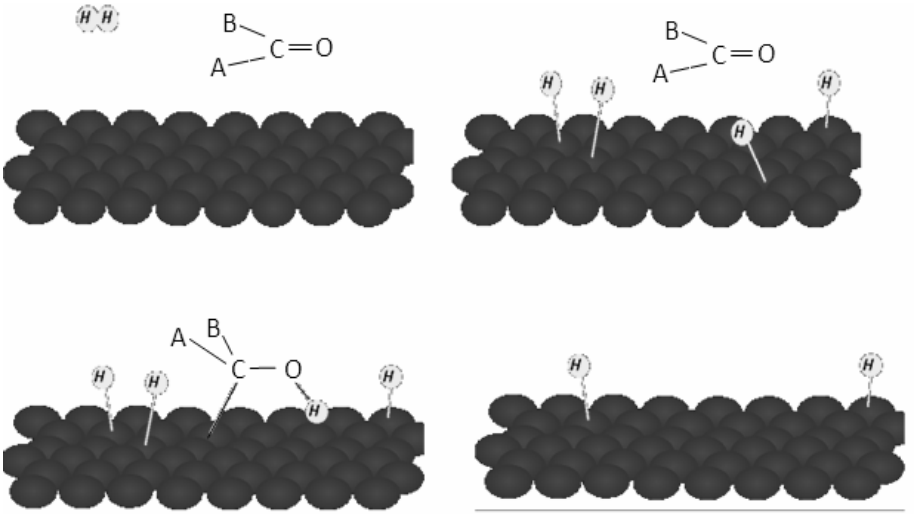


Fig. 2: Activity in the hydrogenation process.

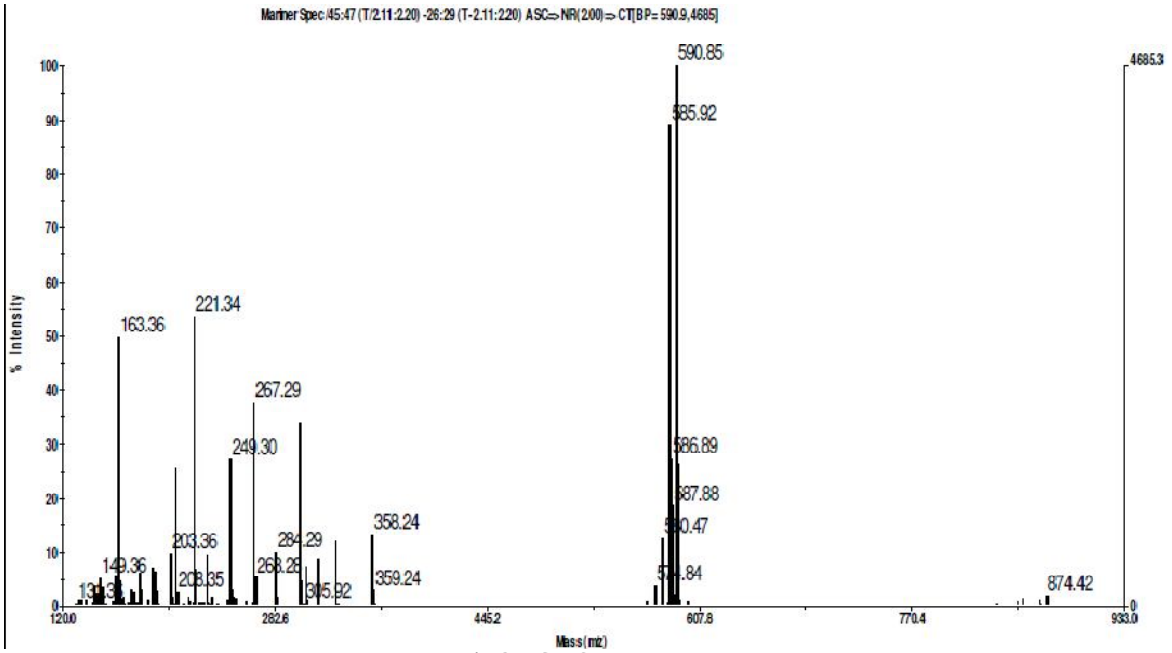


Fig. 3: LC-MS spectroscopy.

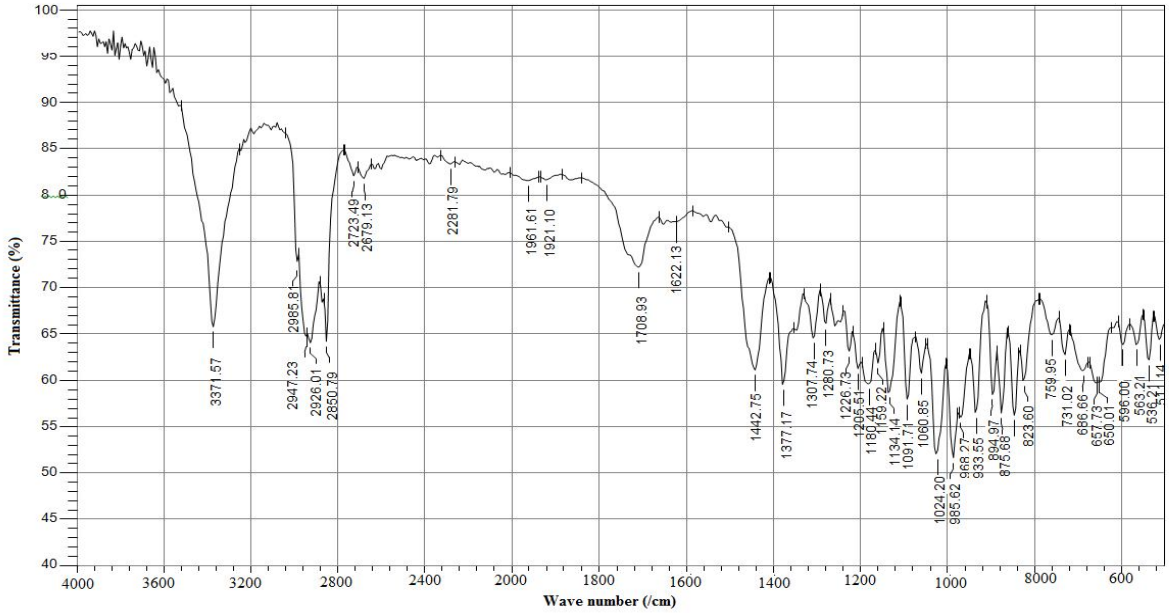


Fig. 4: Infrared measurements spectroscopy for dihydroartemisinin.

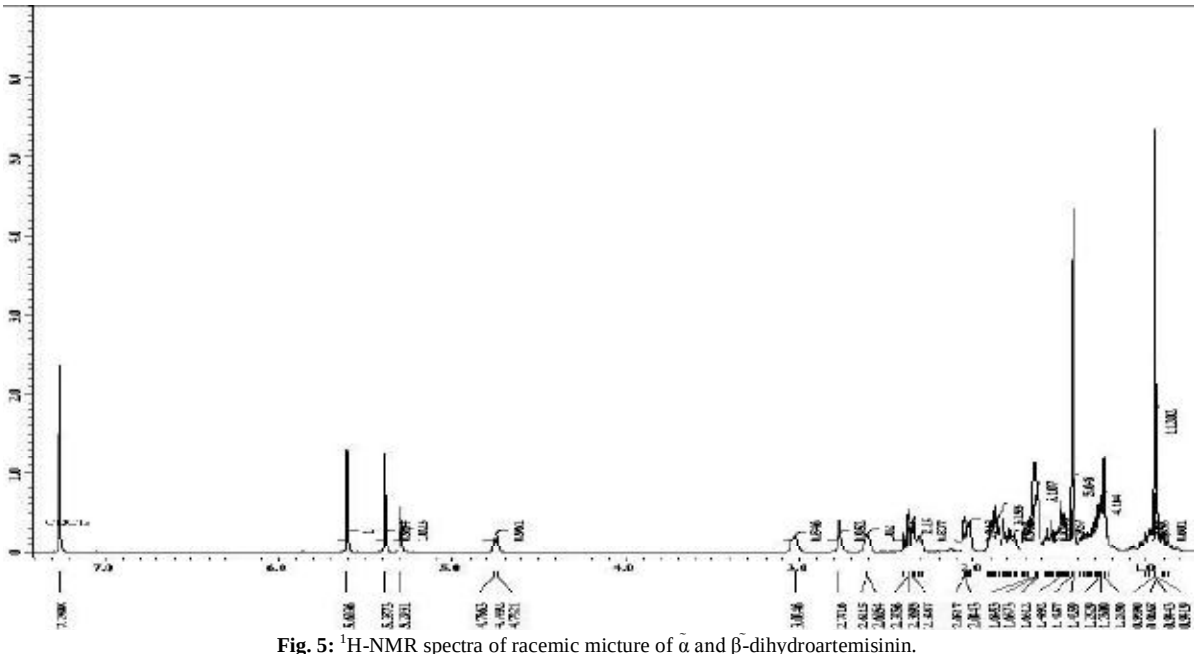


Fig. 5: ^{1}H-NMR spectra of racemic micture of α and β-dihydroartemisinin.

ACKNOWLEDGMENT

The authors wish to acknowledge to colleagues of the Division of Process Technology and Catalysis, Research Center for Chemistry, Indonesian Institute of Sciences, for their important assists during conducting the research in this laboratory.

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How to cite this article:

Leonardus B.S.Kardono, T. Wikara, Harmita, and S.Tursiloadi. Synthesis of Dihydroartemisinin using Ni/Tio_2catalyst Prepared by Sol Gel Method. J App Pharm Sci, 2014; 4 (01): 001-008.