STUDY REACTION OF THE TRANS-\textit{p}-COUMARILPIRROLYDIN COMPOUND FROM \textit{p}-COUMARIC ACID

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\textbf{Abstract.} This study aims to obtain the most effective of reaction condition to convert the carboxylic functional group into the amide functional group. The reaction was done through two methods; direct conversion and indirect conversion (by esterification and amidation). Qualitative data analysis used Infra-Red (FT-IR), spektroskopi \textsuperscript{13}C-NMR, and \textsuperscript{1}H-NMR spectroscopy. The results showed that the target compound could be obtained by indirect conversion, whereas the direct conversion could open the double bond C=C olefinic. The amidation of pirrolydine through indirect conversion gave an unexpected product in the form of unsaturated aliphatic carbon compound

\textit{Keywords:} Amidation, Esterification, Ethyl \textit{p}-coumaric, \textit{p}-Coumaric Acid, trans-\textit{p}-coumarilpirrolydin.

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INTRODUCTION

Secondary metabolites are chemical source that will never run out, as a source of innovation in the discovery and development of new drugs or to support a wide range of industrial interest (Atun, 2005). In addition, the discovery of bioactive compounds that are the starting point to obtain chemicals that can be used to treat a new disease or a substitute for common drugs (Ersam, 2006), including the development of anticancer drugs.

The discovery of new compounds must start from the stage of isolation that requires a number of medicinal plant materials are great and sometimes the amount of active substance obtained is very small; while some plants have retarded growth. Therefore, the development of isolated compounds that have biological activity is very important to be used as a reference compound in the synthesis of new compounds as potential drugs.

One of the active compounds was isolated from the root bark of medicinal plants (Kleinhovia hospita Linn.) is p-coumaric acid or p-hidroxicinamic acid. The isolated compound p-coumaramide very little (± 1.6 ppm), and based on testing of shrimp Artemia salina, p-coumaramide compounds showed fairly high activity (LC₅₀ = 180.53) so it can be potentially as anticancer (Elias, 2008). Therefore, Firdaus et al. (2009) synthesize compounds p-coumaramide of p-coumaric acid, then a test of tumor cell leukemia P-388. Test results against Leukemia Tumor Cells P-388 showed p-Coumaramide compounds have interesting biological activities, with IC₅₀ = 44 μg/mL. According to Anderson (1990), the compound has potent anticancer activity if the IC₅₀ value of 20 μg/mL. Therefore, the compound p-coumaramide basic framework to get more active compounds.

Increased bioactivity of compounds p-coumaramide can be done by holding the molecular structure modifications (Tang, 2005). Accordingly, Eden et al. (2011) have synthesized two derivatives using p-coumarimide Tang (2005), the N-N-diethyl-p-coumaramide (IC₅₀ = 23.50 μg/mL) N-propyl-p-coumaramide (IC₅₀ = 53.56 μg/mL) and p-piperidinyl compounds coumaramide (IC₅₀ = 5.34 μg/mL). The results of the study Zavery et al. (2010), two piperidine alkaloid compound that is pipernonalin and piperoxtadekalidin isolated from Piper longum, both showed insecticidal activity against five species of pest arthropods. Pipernonalin also have potential activity against Puccinia recondita as a fungicide to control values by 91% and 80% respectively at a concentration of 0.5 and 0.2 mg/mL. Ether extract of the roots of the plant Piper Ningrum L. containing piperine alkaloid amide, (E)-1-[3',4'- (methylenedioxy) sinamoil]. Piperidine showed cytotoxic activity against HL60 (Human promyelocytic leukemia cells) with IC₅₀ values of 9.8 μg/mL (Ee et al., 2010). Primary amide group of compounds contained in the p-coumaramide will be modified into a tertiary amide group such as pyrrolidinyl-p-coumaramide. In terms of structure, these compounds are expected to be much less polar making it easier for these compounds pass through the cell membrane lipid containing compounds (Shargel and Yu, 1985). This process can result in the concentration of the compounds absorbed by the cancer cells become larger so that the activity of the compound will be higher.

Through analysis retrosintesis, p-coumaric compounds can be used as the starting material for the synthesis of several compounds derived p-coumaramide targets. p-Coumaric acid crystalline solid, slightly soluble in water, but very soluble in ethanol and diethyl ether. p-hidroksisimamat acid abundant in plant moal, especially on the type of plants that can be consumed as whole grains, vegetables, fruits, and garlic (Kyli et al., 2008). These compounds can be produced biologically from glucose via deamination of aromatic amino acid
L-tyrosine and L-phenylalanine by the enzyme phenylalanine/tyrosine ammonia lyase (PAL/TAL) (Xue et al., 2007). Deamination of L-phenylalanine produce trans-cinnamic acid (CA) subsequently undergo hydroxylation at the para position to produce p-hidroksisiammat (Vannelli et al., 2006) and may reduce the risk of colon cancer by outlining the structure of carcinogenic nitrosamines (Camarero et al., 2008).

Some derivative acid p-coumaric compounds including suberin which serves to maintain viability in a critical condition in all plant tissues (Bernards et al., 1995), kafeat acid (CaA), chlorogenic acid (ChA), sinapatic acid (SA), ferulic acid (FA), and p-kumarat acid (CoA) which acts as an antibacterial (Herald and Davidson, 1983) and can reduce nitrite and inhibit the formation of nitrosamines (Kikugawa et al., 1983).

If using p-coumaric acid as the starting material carboxylic group will converted into amide groups. Conversion reaction can be used in this research is the direct conversion reaction of carboxylic acid groups into amide groups, using boric acid catalyst (Tang, 2005). According to Tang (2005), boric acid is a very effective catalyst for the formation of amides used directly because it can minimize the formation of byproducts. In most cases, the use of 1-5% mole H₂BO₃ sufficient to ensure complete conversion. For example, the synthesis of N-benzyl-4-phenylbutiramid of 4-phenyl butyric acid and benzyl amine using 1% boric acid catalyst, generating rendament 88-91%.

One of the things that are also a consideration in the conversion reaction of carboxylic groups into amide is the presence of a double bond at position α, β on p-coumarat. Using of limited reagents can lead to inefficient reaction and the use of excess reagent and reaction time and temperature handling can lead to the formation of byproducts as a result of substitution at the β-position. How that can be taken to avoid the occurrence of adverse reactions is to set the conditions such as the reaction between the substrate and the mole ratio of reactants, temperature and reaction time. Hence, use an indirectly conversion method through esterification and amidation at low temperatures remains to be done to compare the results of the reaction of both methods.

Through the selection of methods and appropriate reaction conditions, the expected information obtained in this study for the development of the synthesis of p-couaramide derivatives is trans-p-kumarilpirrolydin that may have bioactivity against P-388 Leukemia cells.

RESEARCH METHODS

Material

The materials used in this study were p-coumaric acid p.a, pyrrolidine p.a, H₃BO₃, concentrated H₂SO₄, AlCl₃, 7733 and 7734 silica gel, N,N-Dimethylformamide p.a, ethanol p.a, glass wool, TLC plate, distilled water, chloroform p.a, ethyl acetate p.a, n-hexane p.a, acetone p.a, Whatmann 42 filter paper, and capillary tube.

Instrument

The tools used in this study is three-neck round bottom flask, condensor, thermometer 300 °C, analytical balance, heating mantle magnetic stirrer, Deans strak trap, measuring melting points, UV lamps, rotary evaporator, column chromatography, Buchner funnel, FTIR spectrophotometer, NMR spectrometer, and glass tools commonly used in laboratories.

Procedure

Synthesis of Trans-p-coumarilpirrolydin

0.5 g (3 mmol) p-coumaric acid was inserted into the three-neck round bottom flask. Then added with 0.037 g (0.6 mmol) of boric acid and 30 mL of DMF. The solution was added 0.76 M1 (3 mmol) pyrrolidine then refluxed at a temperature of 130 °C for 4 hours. Further refluxing solution has cooled to room temperature, then added with 10 mL of hot distilled
water and then extracted with diethylether (4 x 30 mL). Diethylether extract obtained re-extracted with water (3 x 30 mL) to remove residual DMF is possibility mixed with diethylether. The organic phase was dried with anhydrous Na₂SO₄, then evaporated and then fractionated by gravity column chromatography using chloroform eluent 100%. Fractions that have the same Rf were combined, then evaporated again until a yellow solid. This solid was crystallized or recrystallized using chloroform: n-hexane to obtain a white solid. Furthermore, the purity test by measuring the melting point and TLC analysis of the compounds synthesized. The pure compound was analyzed by FTIR spectroscopy, and ¹H - NMR.

Esterification of p-Coumaric Acid is Using H₂SO₄ Catalyst

0.5 grams (0.003 mol) p-coumaric Acid inserted into the three-neck round bottom flask was added 20 mL of ethanol, 1 mL H₂SO₄ catalyst (16% of the concentration of p-coumaric acid), and 30 mL of benzene. The Solvent mixture was refluxed at a temperature of 64 °C for 12 hours and then neutralized with K₂CO₃ 15%. Once neutralized, the mixture was washed with distilled water (3 x 20 mL). The organic phase obtained is dried with anhydrous Na₂SO₄ and then evaporated until a yellowish white solid. Furthermore, the solids crystallized and recrystallized using chloroform: n-hexane to obtain white crystals with a melting point of 133-134 °C.

Amidation Of Ethyl p-Coumaric with pirrolydin

0.5 grams Ethyl p-coumaric inserted into the three-neck round bottom flask and then mixed with 30 mL of acetone, 0.6 mL of pyrroldine, and 0.4 grams of AlCl₃, and then stirred at 20 °C for 9 hours (control by TLC test every 3 hours). After stirring, the solvent mixture was neutralized with 1 M HCl subsequently extracted with chloroform (3 x 30 mL). The organic phase was dried with anhydrous Na₂SO₄ and then evaporated until a blackish brown viscous liquid. Further fractionated with Gravitation Coloum Chromatography (GCC) to produce a fraction A-G (fraction A (1), fraction B (2-6), fraction C (7-8), fraction D (9-16), fraction E (17-19), Fraction F (20-23) and fraction G (22-28). fraction E (17-19) in GCC back to obtain 3 fractions, E1 - E3, E4 - E9 and E5 - E18. E4 - E9 Only a fraction of which can be crystallized and recrystallized (benzene and n- hexane) to obtain 0.035 g of white crystals with a melting point of 125-126 °C. Further crystals were analyzed with FT-IR spectrophotometry, H NMR, and C NMR spectroscopy to determine the structure of the target compounds synthesis.

RESULTS AND DISCUSSION

Synthesis of trans-p-coumarilpirrolydin using Boric acid As catalyst

Research synthesis of one the p-coumaramide derivatives is trans-p-coumarilpirrolydin been done by reacting with p-coumaric using boric acid catalyst. The reaction is expected to occur from this mix is the substitution reaction between the hydroxyl group (-OH) of the carboxylic acid with a secondary amine group resulting in the conversion of pyrroldine carboxylate groups into amide groups (Figure 1).

![Reaction diagram](image)

**Figure 1.** Reactions are believed to occur between p-coumaric with pyrroldine using boric acid catalyst

The mechanism of the formation of amide compounds that may occur is shown in Figure 2.
The synthesis reaction takes place at 130 °C under reflux conditions for 4 hours. Reflux time control is obtained by using TLC every 2 hours (Figure 22). Based on the results of the TLC analysis viewed under UV light at λ 254 nm, it is known that at 2nd hour the reaction product had formed, but there is still the same spot with p-coumaric (limiting reagent). After the 4th hour, the spot of limiting reagent is no longer appears indicating that the acid p-coumaric has completely reacted. Rf value of the product is higher than the standard Rf values, it indicates that the product compound has a lower polarity than the reactants. Reaction mixture before refluxing colorless whereas after refluxing the mixture is yellow.

The addition of cold distilled water to the mixture of reaction products is done to avoid the occurrence of adverse reactions; because of the results of the initial preparation, to mixing distilled water at room temperature causes the reaction products give off heat. Furthermore, the addition of distilled water also serves to increase the polarity of DMF, thus simplifying the process of extraction with diethyl ether. DMF and polar compounds will be attracted to the water layer while nonpolar compounds that are going to be attracted to the layer of diethyl ether. Extraction of samples with common organic solvents such as hexane, chloroform and benzene to attract organic compounds in the mixture cannot be done because these solvents are also mixed with DMF.

Based on the results of GCC chromatograms, it can be seen that the resulting product is relatively unstable, at which time the TLC test was stopped shortly after the reflux; the mixture has only one spot, but after the slow evaporation begins to form new spots. Similarly, when the fraction 4-8 dried with anhydrous sodium sulfate back then evaporated until a thick yellow liquid (soluble in chloroform), and then crystallized with chloroform: acetone obtained white solid weighing 0.0972 grams. The only one spot shortly after crystallization, after recrystallization again with chloroform: n-hexane at room temperature, a white solid was obtained crystalline form is easily soluble in acetone, slightly soluble in ethyl acetate and insoluble in chloroform. TLC results indicate the presence of compounds having a spot of Rf value lower than the standard Rf.

Spectroscopic analysis of the structure of the compounds synthesized have been carried out using 1H-NMR and FTIR spectrophotometer. In the FTIR spectra (Table 1) indicate the presence of C = O amide group in compound synthesis products are peak 1654.92 cm⁻¹ which has a lower intensity than the absorption frequency of the C = O group of carboxylic acids at 1687.71 cm⁻¹. Double bond character of the carbonyl carbon and oxygen is reduced due to the delocalization of electrons pairs belonging to the nitrogen atom attached to the carbonyl carbon. This indication is reinforced by the uptake of CN stretch at 1512.19 cm⁻¹, which is characteristic of tertiary amides and non-absorption frequency of C-OH groups of carboxylic reaction products. In addition, the presence of a strong absorption band at 2930.23 indicates that the product has the CH aliphatic compounds, which are not found in the FT-IR spectrum of p-coumaric acid. Uptake of CO bond at 1325.10 cm⁻¹ and 1313.52 cm⁻¹ derived from carboxylic p-hidroxicinamic acid compound also does not appear in the spectra of compounds synthesized products. However, the frequency of uptake in the absence of products in the area of 1627.92 cm⁻¹
indicates that the C = C bond of olefins undergo breakage forming a single bond, it is also reinforced by the absence of absorption frequency of 977.91 cm\(^{-1}\) as a trans 1,2-substituted the compound p-coumaric acid.

Solving the double bond can also be caused by using the reflux temperature is too high. According to Hart (2002), the conversion of a double bond into a single bond is caused by the energy (temperature) is sufficient to break the bonds of \(\pi\) (pi). Bond strength on bond \(\pi\) (pi) is smaller than the sigma bond (\(\sigma\)).

Analysis by \(^1\)H-NMR spectrometer using the compounds synthesized have also been carried out. Absence of signal in the area of chemical CO\(_2\)H shear above 11 ppm in \(^1\)H-NMR, indicating that the compound is not a carboxylic acid compound. The existence of the aromatic group can be identified by the presence of the signal region \(\delta\) 6.2328 ppm to 6.7815 ppm. However, the presence of the H-NMR spectra were experiencing broading the base line, and the presence of protons that are not expected to lead to the elucidation of the structure of the compound product of this reaction is not passed to the C-NMR.

One cause of the existence of protons is undesirable instability resulting product, which is based on the observation, shortly after purification, through crystallization/recrystallization with chloroform: n-hexane, the product obtained was a white solid, soluble in chloroform, and have a higher spot than the standard, even though some days are stored, the compound changes color from white to brownish yellow and only soluble in acetone, this situation is also reinforced by the presence of spots that cannot be eluted using chloroform eluent.

The instability of these compounds is probably caused by the presence of alkyl groups attached to the carbonyl group so that the nature of the acidity of phenol increased acidity of phenol group can be shown by the conjugation system that can happen compound trans-p-coumarilpyrrolidin (Figure 3).

### Table 1. Comparison of FTIR spectral data with a standard product (p-coumaric acid).

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Frequency of absorption at the compound product (cm(^{-1}))</th>
<th>Frequency of absorption at the p-coumaric acid compound (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH-alifatic</td>
<td>3018,31</td>
<td>3026,31</td>
</tr>
<tr>
<td>stretching C=C</td>
<td>-</td>
<td>1627,92</td>
</tr>
<tr>
<td>olefin bending</td>
<td>1377,17</td>
<td>1377,17</td>
</tr>
<tr>
<td>O-H</td>
<td>3375,43</td>
<td>3377,36</td>
</tr>
<tr>
<td>C=O</td>
<td>1654,92 (C=O amide)</td>
<td>1687,71 (C=O carboxilic acid)</td>
</tr>
<tr>
<td>aromatic C=O</td>
<td>1608,63 &amp; 1504,48</td>
<td>1597,06 &amp; 1508,33</td>
</tr>
<tr>
<td>aromatic C-N</td>
<td>1512,19</td>
<td>-</td>
</tr>
<tr>
<td>substitution</td>
<td>829,93</td>
<td>829,93</td>
</tr>
<tr>
<td>para aromatic</td>
<td>-</td>
<td>977,91</td>
</tr>
<tr>
<td>Trans 1,2-</td>
<td>-</td>
<td>1325,10 and 1313,52</td>
</tr>
<tr>
<td>substitution</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C-O (oxi-carbon)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3.** Conjugation of electrons that can occur in the compound trans-p-coumarilpyrrolidin.
Esterification Acid Catalyst is p-Coumaric Using H$_2$SO$_4$

The resulting reaction product in the form of white crystals, soluble in chloroform, has a melting point of 133-134 °C. The results of the analysis of FTIR, H-NMR and C-NMR compound esterification product is in conformity with the data of FTIR ethyl p-coumaric that have been synthesized by Eden et al., (2013).

Compounds Synthesis of trans-p-Coumarilpyrrolidin Through Esther Amidation

Amidation reaction between ethyl p-coumaric with pyrrolidine with AlCl$_3$ catalyst (Figure 4) has been carried out through the method of stirring at a temperature of 15-20 °C. Ethyl p-coumaric soluble in chloroform and acetone, but use of AlCl$_3$ catalyst using chloroform is not optimal because it does not dissolve in the solvent, so that the mixing is done by using acetone. Stirring was stopped after the formation of the optimal product, the TLC test every 3 hours to find the product formation. At the 9th hour, the results of the chromatogram shows the same after the formation of the optimal product, the TLC test every 3 hours to find the product formation. At the 9th hour, the results of the chromatogram shows the same spot with the standard (p-coumaric acid) the less and not much different from that spot 6th hour.

![Figure 4. Expected reaction between p-ethyl coumaric with pyrrolidine](image)

In the process of this amidation reaction, the reaction mixture having a pH = 12, therefore neutralized with HCl 1 M solution for easy excess pyrrolidine extracted into the water phase. In addition, the system generates reaction byproducts such as ethanol that required extraction 3-4 times. Based on TLC analysis using chloroform eluent, giving the product a high Rf value (Rf 0.45), indicating that this compound is soluble in the eluent, so that extraction is done by using chloroform. The use of anhydrous sodium sulfate is used to bind the water that is likely tied to the chloroform extract. The existence of water-free organic phase can facilitate concentration through evaporation at a temperature of 46 °C.

The evaporated in the form of a viscous fluid blackish brown. This fluid showed 5 spots on the chromatogram (chloroform eluent: n-hexane (9:1)) so do fractionation with GCC with the eluent. GCC fractionation results shows there are 9 fractions (fraction A (1). B (2-6), C (7-8). D (9-16). E (17-19). E (20-23) and F (22-28) fractions.

B and E fraction successfully purified, but the results are the dominant fraction E. Fraction E yellow needle crystals formed after 24 hours of settling. After the TLC test, these crystals showed 2 spots on the chromatogram (elucent: chloroform) and subtle so that fractionated crystallization back through KKG to the E4-E9 fraction with one spot which gives the value of Rf 0.24 (elucent chloroform: hexane (7:3)). Crystallization and recrystallization fraction E4-E9 using benzene: hexane yield white needle crystal 0.035 grams (5.6360 rendamen%) with a melting point of 125-126 °C. Based on the purity test with 3 kinds of eluent n-hexane: chloroform (6:4, 4:6 and 1:9), respectively chromatograms the Rf value of 0.35; 0.4; and 0.42.

White crystals with a melting point of 125-126 °C obtained have been analyzed by FT-IR spectrophotometer. In the FT-IR spectrum, there is strong absorption at wave numbers 1701.22 cm$^{-1}$ derived from the carbonyl. The existence of absorption at 1637.56 cm$^{-1}$ and 980.55 cm$^{-1}$ indicates the presence of C=C double bond in the trans configuration olefinic inside the compound. Nonetheless, the absence of absorption at about 1600 cm$^{-1}$ and 1500 cm$^{-1}$ indicates
that the structure of the compound has no aromatic group.

H NMR spectroscopic analysis showed a singlet proton signal at region δ 1.03 ppm, δ 1.07 ppm, and 1.58 ppm, respectively derived from the 3 methyl group (-CH₃). Singlet proton signal at region δ 2.33 ppm and 2.19 indicate that the compound has metin protons (-CH) and methylene (CH₂), as well as the doublet-doublet proton signal at region δ 1.74 ppm (J = 14.25) and δ 1.67 (J = 14.25) comes from the protons attached to the carbon trans olefin. Absence of a proton signal at δ 6-8 ppm region indicates that the compound is not targeted synthesis of aromatic compounds.

C NMR spectrum of the products of compounds having 8 carbon atoms, where in the carbon atom at regions δ 210, 74 ppm derived from the C = O, three metin carbon at δ 53.89 ppm, δ 52, 98 ppm and 52.04 ppm, 3 carbon each methyl is derived from the signal at region δ 30.73 ppm, δ 32.58 ppm and 35.85 ppm, while the signal at region 44.04 ppm derived carbon in the methylene group (CH₂). Through the analysis of the results of FT-IR, H NMR, and C NMR, it is known that compounds that successfully purified an aliphatic compounds having a carbonyl group and 8 carbon atoms. These compounds are probably derived from side reactions such as aldol condensation of acetone as the solvent Figure 3. Changes in p-ethyl coumaric aliphatic compounds would be likely to occur at low temperatures and the presence of AlCl₃ catalyst. This is because the compound is a compound having an aromatic group. The existence of the resonance energy of aromatic compounds cause the compounds are not able to undergo addition reactions like alkene (Fessenden and Fessenden, 2010). Possible target compounds resulting from the reaction, but in a minor amount. This is reinforced by the presence of nine spots on the chromatogram after fractionation. Selection of other solvents such as chloroform has been done, but generated as a result of a heterogeneous mixture of AlCl₃ and H₃BO₃ catalyst used is not soluble in solvents less polar than acetone. Mechanism of aldol condensation reaction that can occur in acetone is shown in Figure 5.

![Figure 5. Aldol condensation of acetone in the presence of AlCl₃ catalyst](image)

**CONCLUSION**

Reaction between p-coumaric with pyrrolidine through direct conversion method using boric acid catalyst, at a temperature of 130 °C reflux produce an amide compound and occurred opening the C=C double bond of olefinic system.

Results of the reaction between p-coumaric with pyrrolidine through gradual conversion method; esterification and amidation, resulting byproducts are carbon aliphatic compounds.

**REFERENCES**


